Fishmeal replacement by cottonseed by-products in the diet of *Trachinotus blochii* (Lacepede, 1801)

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1. ABSTRACT

A ten weeks study was conducted to determine the effect of complete replacement of fishmeal (FM) using cottonseed meal (CSM) on growth, metabolism, nutritive profile, free gossypol accretion and histology of snubnose pompano, *Trachinotus blochii*. Fish with the average weight of 12.5± 0.5 g were randomly stocked in five treatments each in triplicate. Five isonitrogenous and isolipidic diets with graded level of CSM (0, 8.7, 17.4, 26.0 and 34.7%) as replacement for FM protein (0, 25, 50, 75, and 100%) were formulated and fed to respective treatments. There was significant difference ($P<0.001$) in growth in terms of specific growth rate, daily growth coefficient, growth rate on metabolic body weight and thermal growth coefficient and higher responses were observed in 0CSM group followed by 100CSM group. The feed utilization parameters showed significant difference ($P<0.001$) among the treatments and better feed gain ratio, protein efficiency ratio, protein productive value and feed intake per metabolic body weight were observed in 0CSM group followed by 100CSM. Serum triglycerides, cholesterol, phospholipids and total lipid content showed significant difference among the treatments and observed a decreasing trend as the level of inclusion of FM was decreased by replacing with CSM. Free gossypol accretion of 1.28 mg kg^{-1} (on wet basis) was recorded in muscle tissues of 100CSM group which was below the critical limit set by Food and Drug Administration (FDA). The liver histology revealed that hepatocytes of liver section of 100CSM group were normal with no abnormalities and was comparable with 0CSM. In comparison with control diet, the relative percentage of feed cost reduction of various experimental feeds with graded levels of CSM in the range of 2.96-10.08 %. Effectively, fishmeal can be completely replaced using cottonseed meal with lysine and methionine supplementation in the diet of snubnose pompano without adverse effect on growth, metabolism and general health of the fish.

Cottonseed protein concentrates (CPC) was produced from cottonseed meal (CSM) through isoelectric pH precipitation method and the production protocol was optimized for the maximum yield of CPC. The average yield of CPC was 28.35%. The protein content of CPC was 69.47±1.28% which showed an increase of 64.97% of protein content of CSM used for production. The amino acid profile of CPC was on par with fishmeal for most of the essential amino acids expect for lysine and methionine. The CPC production process significantly reduces the free gossypol content of CSM to the extent of 79.73% which will help to alleviate the adverse

effects of CSM. The results of this study revealed that CPC will be a suitable alternative protein source for replacement of fishmeal in the diet of marine fishes due to its higher digestible protein and superior amino acid profile and lower gossypol content. The growth in terms of weight gain percentage and specific growth rate (SGR) showed significant difference ($P<0.001$) and thermal growth co-efficient (TGC) and daily growth co-efficient (DGC) of snubnose pompano fed with graded levels of CPC to replace protein content of FM in the diet showed significant difference ($P<0.05$) and better performance observed in 0CPC group. The feed conversion ratio (FCR), feed efficiency (FE), protein efficiency ratio (PER), protein productive value (PPV), energy retention efficiency (ERE) and nitrogen retention efficiency (NRE) showed a significant difference ($P<0.05$) among the treatments. The intestinal protease activity showed significant difference ($P<0.001$) among the treatments. Among the treatments higher protease activity was witnessed in 0CPC group and the activity was decreased as the level of inclusion of CPC increased in the diet. There was no significant difference ($P>0.05$) in the α-amylase and lipase activities among the treatments. As the inclusion of CPC increased in the diet, the SOD, CAT and GST levels were gradually increased, and a higher level was recorded in 100CPC group. The reduction in the number of lengthwise aligned absorptive cells with supranuclear vacuolisation (SNV) of the enterocytes in the mucosal folds (MF) was seen in higher inclusion of CPC in the diet. The thickness of lamina propria (LP) has increased with a mild infiltration of inflammatory cells (IC) especially neutrophils and size of goblet cells (GC) slightly increased and not the numbers in the epithelium of fishes fed with higher inclusion of CPC (75CPC, 100CPC) in the diet. Necrotic lesions (N) observed in the microvilli (Mv) seen by detachment of surface epithelial cells from underlying lamina propria of fishes fed with diet containing complete replacement of fishmeal using CPC (100CPC). The second degree polynomial regression analysis of valuable indicators in snubnose pompano fed with varying levels of dietary CPC to replace fishmeal in the diet revealed that 8.29-17.28% is optimal for better growth, digestion and metabolism.

A study was conducted to determine the effect of dietary free gossypol on growth and immunological response of snubnose pompano. Fishes with the average weight of 11.43 ±0.25 g were randomly stocked in five treatments each with triplicates. Five isonitrogenous and isolipidic diets with graded level of free gossypol (0, 0.2, 0.5, 1 and 2 g kg^{-1}) were formulated and fed to respective treatments. Growth in terms of weight gain percentage, specific growth rate (SGR)

and average daily growth (ADG) did not show any significant difference (P>0.05) among the treatments. The feed utilization in terms of FCR, FE and PER showed significant difference among the treatments (P<0.05). There was no significant difference (P>0.05) in the hematological response among the treatments. Lipid biochemical profile in terms of total lipid, triglycerides and very low-density lipoprotein showed significant difference (P<0.001) among the treatments. However, serum cholesterol, phospholipids, high density lipoprotein and low-density lipoprotein levels did not show significant difference among the treatments (P>0.05). The acetylcholinesterase (AChE) activity and respiratory burst activity from different treatment groups of *T. blochii* juveniles showed significant difference (P<0.001) among the treatments and showed a decreasing trend as the level of gossypol inclusion increased in the diet. Serum glucose level showed an increasing trend and liver glycogen showed a decreasing trend as the inclusion of gossypol increased in the diet. The phagocytic activity and phagocytic index showed significant difference (P<0.001) among the treatments and higher response was noticed in 0.2G group. After experimental challenge with *Vibrio parahaemolyticus* better relative percentage of survival was observed in 0.2G group and followed by 0G group. No significant difference in survival and mortality rate of snubnose pompano among the treatments. Based on the second-degree polynomial regression analysis of serum lipid biochemical parameters such as total lipids and high-density lipoprotein levels it could be concluded that snubnose pompano can tolerate up to 1.2 g of free gossypol kg^{-1} diet without adverse effects on general health.

2. INTRODUCTION

Global fish production has been increasing for the past six decades and during 2018, the aquaculture alone contributed 82.1 Mt which is 45.99 % of total fish production (FAO, 2020). Coastal aquaculture production contributed 30.8 Mt to the total aquaculture production of 82.1 Mt during 2018 with the maximum contribution of 88.69% from Asian continent (FAO, 2020). As the fish production increases, fish feed production is also increasing tremendously. Even though aquafeed production increases, cost effective feed is not available in the market, especially for carnivorous marine fishes. The main reason for such situation is due to the higher level of inclusion of fishmeal (FM) and fish oil in the diet of carnivorous marine fishes. Thus, fishmeal became one of the most indispensable components in marine fish feeds and its requirement is increasing exponentially. Fishmeal production is almost static around 7 million

tonnes, except during *El Nino* periods where the fishmeal production goes even lower (4.5-5 Mt) (Hardy and Tacon, 2002; Hardy, 2010). About 10% of the total global fish production is utilized for FM production, in which 73% is used by aquaculture industry alone (Shepherd, 2012). Further, the formulated fish feed requirement is swelling every year due to the transition from extensive, semi-intensive farming practices to more and more intensive farming. As there is no scope to escalate the FM production in future and the FM price is increasing steadily. The increased demand of FM coupled with rise in prices led to the search of alternative protein sources suitable for the replacement of FM to the maximum extent possible.

Most of the aquafeed manufacturers have started reducing the inclusion of FM at least by 5-10% in almost all species under culture worldwide (Hardy, 2006). The utilization of FM in fish feeds has plummeted to 19 % in 2005 to 13 % in 2008, and it has been anticipated that it will further tumble down in future (Van Huis et al., 2013) through the effective utilisation of alternative source ingredients. Therefore, utilization of FM in marine carnivorous fish feeds has started declining over the years. Thus, the main challenges ahead for the aquafeed industry are to reduce the inclusion rate of FM in feeds and to source out economically viable and environment friendly alternative ingredient sources (Agbo, 2008). Hence, it is high time for us to protect the declining natural resources (fishmeal and fish oil) and make it available for human consumption.

In this context, fishmeal replacement is tried with several plant source protein ingredients both commercial and non-conventional sources. Among the several feed ingredients, agricultural by-products or plant protein sources are widely available, renewable and reasonably priced. Therefore, the use of plant protein sources in aquafeeds was considered as potential alternatives to fishmeal. Among the different plant source proteins, cottonseed meal (CSM) is one of the most promising alternatives to marine source ingredients due to its high protein content (42-45%), palatability, wide availability at reasonably low price (Li and Robinson, 2006). Like other ingredients of plant origin, cottonseed meal also contains some anti-nutritional factors (ANFs) such as gossypol, cyclopropene fatty acids, phytic acid and tannin. In aquafeeds, CSM is used to replace soybean meal and FM due to its better palatability and nutritional quality (Li and Robinson, 2006). Among the ANF's of CSM, free gossypol (FG) is a major one and is a phenolic aldehyde that causes anorexia, anemia, pathological changes in liver and kidney, and abnormal gametogenesis (Yue and Zhou, 2008; Yan et al., 2014). Recent days, the free gossypol content of CSM was reduced by selective breeding programs of glandless varieties with low FG, microbial

fermentation, extrusion process and inclusion of lysine and ferrous sulphate during the extrusion process or during feed production (Zhang et al., 2007; Bu et al., 2017a). The cottonseed meal was examined for many commercial important fish species such as rainbow trout (Rinchard et al., 2003; Luo et al., 2006), silver crucian carp (Cai et al., 2011), turbot (Bian et al., 2017), black sea bream (Sun et al., 2015), Ussuri catfish (Bu et al., 2017b) and black sea bass (Anderson et al., 2016) to determine the suitable proportions of inclusions as alternatives to fishmeal.

Removal of anti-nutritional factors from feed ingredients is cumbersome and most of the methods employed for eradication of anti-nutritional factors in small scale are not suitable or less appropriate or least effective in commercial scale. Therefore, it is a strategy to separate the protein content from the agricultural by-products rather than amelioration of anti-nutritional factors. In this context, production of protein concentrate or protein isolate from cottonseed meal seems to be a viable option which will increase its protein content, improve amino acid profile, protein digestibility and reduce anti-nutritional factors and fibre content. Several methods are currently employed for protein isolate or concentrate preparation. Among the methods available, the best method will be selected and standardized for cost effective protein isolate/concentrate production from cottonseed meal to increase the protein content and also to reduce the level of gossypol content. The aim of the present work is to elucidate the potential for safe use of cottonseed derived feed ingredients such as cottonseed meal, cottonseed protein concentrate in marine food fish.

Among the different species of mariculture interest, snubnose/ silver pompano is one of the commercially important species because of its fast growth, relatively lower protein requirement, tasty white meat and tolerance to wide range of salinity and temperature (Kalidas et al., 2012). The shape, colouration and meat quality of this fish is comparable with silver pomfret. Snubnose pompano is caught sporadically in the commercial fishery and hence it is a much sought-after species for human consumption. Thus, the demand can only be met through mariculture. The farming of snubnose pompano successfully established in several Asian-Pacific countries such as Taiwan, Indonesia, China and India and being carried out in coastal ponds and floating open sea cages. Pompano is a fast-moving fish and requires highly nutritive feed to meet the energy requirements like most of the carnivorous species. Therefore, the present study is aimed to replace FM using extruded, low free-gossypol content cottonseed meal or cottonseed

protein concentrate to evaluate the effect on growth, feed intake, metabolism, nutritive profile, free gossypol accretion, hematological, histological response, tolerance to level of free gossypol and relative feed cost in the juveniles of snubnose pompano.

3. REVIEW OF LITERATURE

Aquaculture and mariculture scenario

Globally, aquaculture production continued to increase in the past few decades. During the year 2018, the aquaculture alone contributes 82.1 Mt which is 46.00 % of total fish production (FAO, 2020). The freshwater fish production contributes 51.34 Mt and marine and coastal aquaculture production is 30.76 Mt out of total aquaculture production of 82.1 Mt during 2018 (FAO, 2020). Asian continent registered a maximum of 25.10 Mt of marine and coastal aquaculture production which accounts 81.60% of total global marine and coastal aquaculture. Marine aquaculture or mariculture is the constituent of aquaculture and its production is also increasing steadily every year and during the year 2018, world marine finfish production reached 7.33 Mt (FAO, 2020). In case of marine and coastal finfish production, Asian continent with the production of 4.00 Mt leads which accounts 54.57% of global marine and coastal finfish production followed by Europe and America contributes 1.89 (25.78%) and 1.06 (14.46%) million tonnes respectively (FAO, 2020). In India, marine fish culture is getting attention through culture of cobia, pompano, sea bass, grouper and emperor fish in open sea cages, coastal cages and ponds as well as culture of pompano in low saline coastal ponds in different parts of the country. The successful marine fish culture is accomplished by successful seed production technology for above said fishes and seeds are regularly available and supplied to fish farmers. Due to its infancy, the coastal and marine fin fish culture of India contributes just 90,000 tonnes during 2014 which is only 1.42% of global marine and coastal aquaculture.

Aquafeed and fishmeal replacement

As the fish production increases, fish feed production is also increasing tremendously. Globally compounded aquafeed production has increased as the finfish aquaculture production has increased from 7.6 Mt in 1995 to 53.4 Mt in 2017, at an average rate of 5.7 % per year. Actually, the global fish feed production by commercial manufacturers reached about 51.2 Mt by 2017 for the production of 44.4 Mt of fed aquaculture species and the feed production is expected to rise to 58.85 Mt by 2020 and 73.15 Mt by 2025, respectively (Tacon, 2020). For the production of 44.4 Mt of fed aquaculture species, the total feed requirement can be projected as

66.6 and 88.8 Mt if the FCR is 1.5 and 2.0 respectively and thus, the gap in feed production from the commercial manufacturers (51.2 Mt) were bridge up by farm made feeds/ mashes which accounts at least 15 to 38 Mt. India contributes just 1 Mt which accounts 2.43 % of world compound feeds during 2014 (Gilbert, 2015).

Aquafeeds contains higher amount of fishmeal (FM) than feeds of other terrestrial animals. The inclusion level of fishmeal in aquafeed varies according to the feeding nature whether carnivorous or omnivorous and the pattern of utilization of nutrients (Martin, 1999). The cost of carnivorous marine fish feeds is very high due to the higher level of inclusion of marine source ingredients such as fish meal, shrimp meal, krill meal, squid meal and fish oil in the diet. Among the several marine ingredients fishmeal and fish oil are predominantly used as a protein and lipid source respectively, and became one of the most indispensable ingredients in marine fish feeds. The fish meal requirement is growing exponentially, and the fish meal production is almost static around 7 Mt except during *El Nino* periods where the fish meal production will go down even lower (4.5-5 million tonnes) (Hardy and Tacon, 2002; Hardy, 2010). About 10% of the total global fish production is utilized for fish meal production, in which 73% is used by aquaculture industry alone (Shepherd, 2012). Unfortunately there is no scope to escalate the fish meal production in future. Due to these reasons the fishmeal price is increasing steadily.

Most of the aquafeed manufacturers have started reducing the fishmeal inclusion at least by 5-10% in almost all species under culture worldwide (Hardy, 2006). Therefore, the fishmeal utilization in aquafeed during 2002 was 2.21 Mt which accounts 31.46% of total fishmeal produced in the same period for the production of 15.79 Mt aquafeed that accounts 14.03% fishmeal inclusion in feed (Pike and Barlow, 2003). But, they have predicted about 1.74 Mt fishmeal may required to produce 32.27 Mt of fish feed during 2010 which is due to the reduction in the utilization of fish meal in aquafeed. The feed requirement and demand will increase every year which is due to the transition of extensive, semi-intensive farming practices to more and more intensive farming and intensive farming solely depends on nutritionally balanced complete feed.

As FM is the scarcest ingredient due to its decreased supply, high demand and price; there is a search for an alternate ingredient to replace FM in marine fish diets (Fournier et al., 2004). Therefore, in the recent times, utilization of fishmeal in marine carnivorous fish feeds has declined over the past decades. Thus, the main challenges ahead for the aquafeed industry are to

reduce further, the inclusion rate of fishmeal in aquafeeds and to source out economically viable and environmental friendly alternative ingredient sources (Agbo, 2008). The utilization of fishmeal in fish feeds has plummeted to 19 % in 2005 to 13 % in 2008, and it has been anticipated that it will further tumble down in future (Van Huis et al., 2013) through the effective utilisation alternative source ingredients. Search for alternatives to replace fishmeal is still going on. Hence, it is high time for us to protect the declining natural resources (fishmeal and fish oil) and make it available for human consumption.

Alternate feed ingredients

In this context, fishmeal replacement is tried with several protein source ingredients both commercial and non-conventional feed ingredients such as animal by-products (meat and bone meal, blood meal and poultry by-product meals, fishery by-products meal), zooplankton meals, micro algal, bacterial and yeast single cell protein, and wide variety of plant proteins such as oilseeds meals (soybean, ground nut oil cake, cottonseed, canola, jatropha, sunflower oil cake, safflower oil cake, sesame oil cake, copra meal, palm oil kernel meal etc.), legumes, cereal by-products (corn gluten, wheat gluten etc.,) and protein concentrates and isolates by several researchers. Among the several feed ingredients available in the market, agricultural by-products or plant protein sources are widely available, renewable and reasonably priced ingredients (Chakraborty et al., 2019). Therefore, the use of plant protein sources in aquafeeds is considered as potential alternatives to fishmeal (Rana et al., 2009). Among the plant ingredients with good protein content for fish are soybean meal, groundnut oil cake, cottonseed oil cake, mustard/rapeseed oil cake, copra meal, palm kernel meal, lupin meal, guar meal, DDGS, maize gluten, wheat gluten etc. Unfortunately, these plant source proteins are often deficient in certain essential amino acids and boast high amounts of fibre content, complex carbohydrate, non-starch polysaccharides and presence of anti-nutritional factors (ANFs), which cause adverse effects on feed intake, digestion, absorption and leading to reduced growth (Hixson, 2014). The ANFs may also have adverse effects on both their nutritional value and palatability (Kaushik et al., 2004). Hence, a solution to ameliorate the above mentioned issues to an acceptable level for inclusion of agricultural by-products in place of fishmeal in marine fish feeds.

Cottonseed meal

Among the different plant source proteins, cottonseed meal (CSM) is one of the most promising alternatives to marine source ingredients due to its high protein content (42-45%), with better palatability and nutritive value which is slightly lower (85-90%) than that of soybean meal (Forster and Cahloun, 1995) with wide availability and reasonably low price (Li and Robinson, 2006).

The cotton seed kernel is pressed or extracted to produce cottonseed oil (17% yield) which is ranked sixth among the world's edible oils and the residual cotton seed meal (47%) that is used as a very good protein source for fish and other animals (Cheng et al., 2020). Like other ingredients of plant origin, cottonseed meal also contains some ANFs such as gossypol, cyclopropene fatty acids (sterculic acid, malvalic acid and dihydrosterculic acid), phytic acid and tannin. In aquafeeds, cottonseed meal is used to replace soybean meal and fishmeal due to its better palatability and nutritional quality (Li and Robinson, 2006).

Kleemann et al. (2011) replaced soybean meal with cottonseed meal in the diets of Nile tilapia revealed that cottonseed meal can be able to replace up to 50% of soybean meal protein in the diet that accounts 32% of inclusion with synthetic lysine, without impaired growth and intestinal morphology. Rinchard et al. (2002) revealed that cottonseed meal can be used to replace fish meal at the maximum of 50% without affecting the growth and reproductive performance beyond 50 % of replacement have a negative impact on growth of tilapia. In olive flounder, *Paralichthys olivaceus,* Lim and Lee (2008) replaced fish meal up to 40% with mixture of cottonseed meal with iron and phosphorus without any adverse effect on growth. The cottonseed meal was examined for many commercial important fish species such as rainbow trout (Rinchard et al., 2003), channel catfish (Barros et al., 2002), tilapia (Rinchard et al., 2002) etc., to replace either fish meal or soybean meal.

Cai et al. (2011) found that cottonseed meal and free gossypol on allogynogenetic silver crucian carp in different graded levels of cottonseed meal and free gossypol up to 560 g kg^{-1} and 642 mg kg^{-1} respectively did not have any adverse effect on whole body composition, haemoglobin concentration, activities of serum lysozyme, superoxide dismutase and histology of hepatic tissues and midgut. Some research findings reveal that cottonseed meal and gossypol content do not have adverse effect in some fishes and some studies stated that cottonseed meal have deleterious effect on feed intake, growth, feed efficiency in fish, gut health and general

physiology (Lee et al., 2006; Anderson et al., 2016; Liu et al., 2020). Therefore, it is necessary to ameliorate the ill effects of cottonseed meal and gossypol.

Cottonseed protein concentrate

Removal of anti-nutritional factors from feed ingredients is a more laborious work and most of the methods employed for eradication of anti-nutritional factors in small scale are not suitable for commercial scale operations. An alternative strategy is to separate the protein content from the cottonseed meal rather amelioration of anti-nutritional factors. In this context, production of protein concentrate from cottonseed meal seems to be a viable option which will increase its protein content to the tune of 70 % (Martinez et al., 1970), improve amino acid profile, protein digestibility and reduce anti-nutritional factors and fibre content (Gerasimidis et al., 2007; Makkar et al., 2008; Marrufo-Estrada et al., 2013). The use of plant-based protein concentrates is a promising alternative to the use of plant-based protein source ingredients as such (Deng et al., 2006).

Several methods such as iso-electric precipitation, aqueous-enzymatic process and aqueous-alcoholic protein recovery are currently employed for protein isolate or concentrate preparation. Commercially, protein concentrates are prepared by removing soluble carbohydrate fraction as well as some flavor compounds from defatted meal at the isoelectric pH (pH 4.5) of carbohydrates (Guo, 2009). Thus, protein concentrate preparation involves insolubilization of the protein to remove soluble sugars and separation of solid mass by centrifugation which containing mainly proteins and insoluble carbohydrates (Wang et al., 2004). Other than soybean meal, the preparation of protein isolate and concentrate were reported from jatropha (Kumar et al., 2011a; Shamna et al., 2015) rubber seed (Fawole et al., 2016), red seaweed *Kappaphycus alvarezii* (Kumar et al., 2014), sunflower seed meal (Lovatto et al., 2018) and Crambe meal (Lovatto et al., 2018) to enhance its nutritive profile and reduce the anti-nutritional effect.

Zhang et al. (2007) who extracted cottonseed protein concentrate by using an alkaline medium at varying pH values and obtained the cotton seed protein concentrate with the protein content of 70%. Alkali extraction followed by isoelectric precipitation is a suitable method for isolate/concentrate preparation from canola (Drew et al., 2007).

Martinez et al. (1970) who revealed that CSM through further processing, can be prepared as protein concentrate with about 70% protein and protein isolates with 90% crude protein. Wang et al. (2004) found increased protein content in the soy protein concentrate (67.53%)

prepared from soybean meal (54.34%) by the reduction of carbohydrates and fibre contents of source materials. Glencross et al. (2005) reported the total protein content of soy protein concentrate is 65-67 % and canola protein concentrate with 69% protein and lysine content of 3.55% was reported by Drew et al. (2007).

According to Sun et al. (2016), juveniles of *Litopenaeus vannamei* fed with different levels of fermented cottonseed meal to replace fish meal, suggested that up to 50% of fishmeal can be replaced by fermented cottonseed meal without adverse effects on growth and feed utilization of *L. vannamei* and the level going beyond 50% significantly reduces the body ash content and increase the concentrations of total gossypol content.

Snubnose pompano

Among the different species of mariculture interest, snubnose pompano is a commercially important species because of its fast growth, tasty meat and tolerance to wide range of salinity and temperature (Gopakumar et al., 2012). The mariculture of snubnose pompano is successfully established in several Asian-Pacific countries such as Taiwan, Indonesia, China and India. This species can tolerate wide range of salinity and even better growth was achieved at 10 ppt salinity (Kalidas et al., 2012). Therefore, it is widely cultured in open sea cages and coastal ponds.

4. OBJECTIVES

1. To study the effects of cottonseed meal as a substitute to fishmeal on growth, feed utilization, digestive and metabolic enzyme activities, gut and liver health of marine food fish.
2. To develop protein isolate/ concentrate from cottonseed meal and evaluate its utility in the diet of marine food fish to replace fishmeal.
3. To isolate free gossypol from cottonseed meal and study its effects on growth and immunity of marine food fish.

5. MATERIAL AND METHODS

Cottonseed meal

Extruded, low free-gossypol content cottonseed meal (CSM) was procured from Growel Feeds Pvt. Ltd., Andhra Pradesh, India (Fig. 1). The CSM was milled using a kitchen mixer grinder (Preethi Blue leaf™, Chennai, India) to reduce the particle size about 100 µm for feed preparation and to assess the nutritive profile.

Fig. 1: Cottonseed meal used in this experiment

Experimental fishes

Juveniles of snubnose pompano, *Trachinotus blochii* (Lacepede, 1801) were procured from Mandapam Regional Centre of ICAR-CMFRI, Mandapam Camp, Tamil Nadu to Tuticorin Research Centre of ICAR-CMFRI, Thoothukudi in oxygen filled polyethylene bags (Fig. 2).

Fig. 2: Acclimation of experimental fishes prior to stocking in the wet laboratory

On arrival, the pack of juvenile fishes were acclimated to the prevailing water temperature of the wet lab and followed by quarantine measures were carried out (Fig. 3). The salinity of rearing medium was gradually reduced to 15 g kg^{-1} in 15 days duration and maintained at 15 g kg^{-1} for further research.

Fig. 3: Juvenile of snubnose pompano

Setting up of experimental units

The study was conducted in indigenous Re-circulatory Aquaculture System (i-RAS) unit designed for fish nutrition experiment (Fig. 4) (Prabu et al., 2017). After acclimation, the fish were stocked following completely randomized design (CRD) in which fish were subjected to five treatments in triplicate for all the three experiments.

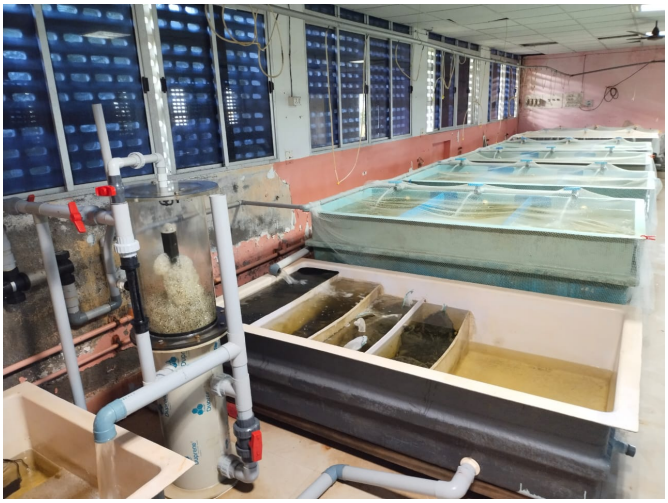


Fig. 4: Experimental unit in which the experiment was conducted

Experimental design

The feeding studies were conducted as according to the experimental designs tabled below.

Table 1: Fishmeal (FM) replacement with graded levels of cottonseed meal (CSM)

Treatments	Conditions	Replicates		
0CSM	Control (Maximum fishmeal inclusion)	R1	R2	R3
25CSM	25% replacement of FM with CSM	R1	R2	R3
50CSM	50% replacement of FM with CSM	R1	R2	R3
75CSM	75% replacement of FM with CSM	R1	R2	R3
100CSM	100% replacement of FM with CSM	R1	R2	R3

Table 2: FM replacement with graded levels of cottonseed protein concentrate (CPC)

Treatments	Conditions	Replicates		
0CPC	Control with FM and no CPC	R1	R2	R3
25CPC	25% replacement of FM with CPC	R1	R2	R3
50CPC	50% replacement of FM with CPC	R1	R2	R3
75CPC	75% replacement of FM with CPC	R1	R2	R3
100CPC	100% replacement of FM with CPC	R1	R2	R3

Table 3: Diets with graded levels of gossypol

Treatments	Conditions	Replicates		
0G	Control diet (No gossypol)	R1	R2	R3
0.2G	Control diet + 200 mg gossypol kg^{-1} diet	R1	R2	R3
0.5G	Control diet + 500 mg gossypol kg^{-1} diet	R1	R2	R3
1G	Control diet + 1000 mg gossypol kg^{-1} diet	R1	R2	R3
2G	Control diet + 2000 mg gossypol kg^{-1} diet	R1	R2	R3

Experimental feed

There were five experimental diets formulated with graded levels of CSM or cottonseed protein concentrate to replace FM in the diet for study 1and 2. The diets were isonitrogenous (40% CP) and isolipidic (6% CF) in nature. As listed in the formulae (Table 4) feed ingredients were weighed accurately, mixed thoroughly, added with required quantity of water, cooked, and passed through laboratory scale motorized pelletizer using 4 mm diameter die and prepared as wet feed strings (Fig. 5). The feed strings were further dried, crushed and sieved through 2 and 3 mm sieve to collect 2 and 3 mm slow-sinking crumble feed (Fig. 6).

Fig. 5: Experimental feeds prior to crumbling and size grading

Fig. 6: Slow sinking crumble feed prepared for this study

Table 4: Feed for cottonseed meal experiment (g kg^{-1})

Ingredients	0CSM	25CSM	50CSM	75CSM	100CSM
Cottonseed meal[1]	**0**	**87**	**174**	**260**	**347**
Fishmeal[2]	**240**	**180**	**120**	**60**	**0**
Soy bean meal[3]	140	150	160	169	176
Squid meal[4]	40	40	40	40	40
Groundnut oil cake [5]	126	129	127.5	129	129
Meat & bone meal [6]	50	50	50	50	50
De-oiled rice bran[7]	73	42.5	15	0	0
Shrimp meal[8]	50	50	50	50	50
Wheat powder[9]	200	190	180	155	117.5
Fish oil[10]	15	15	15	15	15
Sunflower oil[11]	10	10	10	10	10
Vitamin[12,] †	15	15	15	15	15
Mineral[13]‡	15	15	15	15	15
Sodium meta bisulphate[14]	1	1	1	1	1
Methionine[15]	**3.5**	**3.5**	**4**	**4.5**	**5**
Lysine[16]	**3**	**3.5**	**5**	**6**	**7**
Vitamin C[17]	2.5	2.5	2.5	2.5	2.5
Lecithin[18]	5	5	5	5	5
Dicalcium phosphate [19]	5	5	5	5	5
Immunostimulants SWM[20]+NFM[21] +FWM[22]	5	5	5	5	5
Binder[23]	0	0	0	2	4
Butylated hydroxyl toluene[24]	1	1	1	1	1

1Growel feeds Pvt Ltd., Andhra Pradesh; 2Raj Fishmeal and Oil Co., Malpe, Mangalore; 3SakthiSoya, Coimbatore; 4King Fish Products Pvt Ltd., Veraval; 5,7,9&11 Locally procured; 6Arogya Bio Proteins, Vellore; 8KhajaMohideen, Wall Tax Road, Chennai; 10Crude sardine oil from Kiriyathan Trading Co., Narakkal, Kerala; 12Supplevite – M from Sarabhai Zydus Animal Health Pvt. Ltd, Vadodara; 13Agrimin from Vibrac Healthcare India, Pvt. Ltd., Mumbai; 14,18,19&24 Hi-Media, Mumbai; 15Evonik Degussa, Germany; 16BestAmino, CJ Corporation, South Korea; 17Stay – C from DSM Nutritional Technologies, Mumbai; 20&21 Locally collected; 22Collected from Arunachal Pradesh; 23Pega Bind, Bentoli, USA

SWM, Seaweed meal (*Sargassum wightii*); NFM, Neem flower meal (*Azadirachta indica*); FWM, Fishwort meal (*Houttuynia cordata*).

† Composition of vitamin mix (unit kg^{-1}): Vitamin A, 500,000 IU; Vitamin D3, 250,000 IU; Vitamin E, 2000 mg; Vitamin K, 1,000 mg; Thiamin, 100 mg; Riboflavin, 2,000 mg; Pyridoxine,500 mg; Cyanacobalamin, 400 mg; Calcium Pantothenate, 2,500 mg; Niacin, 4000 mg; Biotin,4000 mg; Folic acid 200 mg.

‡ Composition of mineral mix (unit kg^{-1}): Manganese oxide, 500 mg; Potassium iodide, 500mg; Ferrous sulphate, 10 g; Zinc oxide, 1000 mg; Copper sulphate, 250 mg; Cobalt carbonate, 2mg; Sodium selenite, 10 mg; Chromium chloride, 100 mg; Calcium lactate, 250 g; Calcium phosphate (monobasic), 350 g; Magnesium sulphate, 50 g.

Fish rearing

The fishes were fed to apparent satiation twice daily at 10.00 and 16.00 h using the experimental feeds during the feeding trial. The amount of feed consumed by the fish in each tank was recorded daily. Round the clock filtered water circulation was made through indigenous re-circulatory filter assembly with 15 g kg^{-1} saline water and the flow-rate of water in each tank was kept at 5 L min^{-1}. Faecal matter present in the experimental tanks was siphoned out daily and the released water was replenished with 15 g kg^{-1} saline water.

Growth assessment and feed utilization

At the end of the rearing period, the body weight of experimental fish was measured to assess the growth response and feed utilization (Fig. 7). The fish were starved overnight before taking the body weight and to avoid the fecal matter at the time of sampling. Fish were weighed at a precision of 0.1 g using electronic balance. The growth and feed utilization parameters were calculated using standard formulae.

Sampling, blood collection and homogenate preparation

Two fish from each replicate were anesthetized with MS222 at 100 mg L^{-1} water. The fishes were euthanized and liver, gill, muscle, brain and intestine tissue samples were removed carefully. Tissue samples were homogenized with 0.25 M chilled sucrose solution using tissue homogenizer (T18™ model, IKA® India Pvt. Ltd., Bengaluru, India), centrifuged using refrigerated centrifuge (Remi, CM 12 PLUS, India), supernatant collected and stored at -20 °C freezer (Blue Star Ltd., Mumbai, India) until use. A 10% homogenate was prepared and used for the assay of digestive and metabolic enzymes. From all treatments three more fishes were euthanized for the collection of blood and serum from the caudal vein and subsequently used for estimation of whole-body composition (Fig. 8). The fishes were sliced into pieces and oven dried at 105 °C for 6 h until there was no change in the dry weight.

Fig. 7: Assess the weight of the fish for growth estimation

Fig. 8: Blood collection from the experimental fishes

Free gossypol estimation

The free gossypol content of cotton seed meal, experimental diets and tissue samples of experimental fishes were assessed through HPLC (Waters, USA) by the method of Karishma et al. (2016). One gram of sample was added with 50 mL of 70% acetone to which 10 g of glass beads were added and placed in a shaker for 1 h. It was then filtered through Whatmann filter paper No. 1 and 1 mL of filtrate was collected to which 1 mL of N, N di-methylformamide and 1 mL of complex reagent was added. This complete setup was placed in water bath for 30 min at

100 °C. Solution was allowed to cool to room temperature and was diluted to 5 mL by adding 3 mL of N, N di-methylformamide from which 20 µL of sample was analysed by HPLC.

Proximate composition

The proximate composition of feed ingredients, experimental feeds and whole-body fish samples were carried out. The crude protein content (nitrogen × 6.25) was determined by the Kjeldahl method after acid digestion using a Kjeldahl System (FOSS Kjeltec 2300). Crude lipid content was estimated using a Soxhlet System (FOSS Soxtec 2043). Total ash content was determined by incinerating the feeds and tissue samples in a muffle furnace (Kemi Lab Equipment, Kerala, India) at 600 ºC for 4 h (AOAC, 2000; method 942.05) and moisture content was estimated using hot air oven (AOAC, 2000; method 934.01).

Essential amino acid profile of feed and tissue samples

The amino acid profile of experimental feed and whole-body fish tissue samples were estimated using reverse-phase high-performance liquid chromatography (HPLC) (Waters, USA). Samples were hydrolyzed with 6 M HCl at 110 ºC for 24 h. Hydrolysates were added to a C-18 column and amino acids were separated via reverse phase HPLC. Essential amino acids were quantified with a photodiode array detector following post-column derivatization with ninhydrin (Smith, 1997).

Serum biochemical markers

Serum total protein was determined by biuret method (Reinhold, 1953) using the protein estimation kit (Coral Clinical Systems, Goa, India). Albumin was estimated by Bromocresol green (BCG) binding method (Doumas et al., 1971) using kit (Coral Clinical Systems, Goa, India) whereas globulin level was determined by detecting the values of serum total protein with serum albumin. Serum triglyceride was determined by glycerol phosphate oxidase/peroxidase (GPO/PAP) method (Fossati and Prencipe, 1982) using triglyceride kit (Coral Clinical Systems, Goa, India). Serum cholesterol was estimated by cholesterol oxidase/peroxidase (CHOD/PAP) method (Meiattini et al., 1978) using cholesterol kit (Coral Clinical Systems, Goa, India). Phospholipid content was calculated from cholesterol content empirically by the method of Covaci et al. (2006). Serum total lipid content was calculated by the summation of serum triglycerides, phospholipids and cholesterol contents. Serum very low-density lipoprotein (VLDL) level was calculated as per the methods of Friedewald et al. (1972).

Haematological response

Total erythrocyte (RBC) and leucocyte (WBC) were counted in a Neubauer's haemocytometer (Rohem, India) using erythrocyte and leucocyte diluting fluids provided with the kit (Qualigens Diagnostics, Division of Glaxo Smithkline Pharmaceutical Ltd., Mumbai, India), respectively. The haemoglobin level of blood was analysed by following Cyanmethemoglobin method using Drabkins Fluid (Qualigens Diagnostics, India). The absorbance was measured at wave length of 540 nm using a spectrophotometer and the final concentration was calculated by comparing with the standard cyanmethemoglobin sourced from Qualigens Diagnostics, India. The packed cell volume (PCV) or Heamatocrit (Hct %) was determined by the Wintrobe and Westergreen method as described by Blaxhall and Daisley (1973). It was analysed by drawing non-clotted blood by capillary action into microhaematocrit tubes. The microhaematocrit tubes were sealed at one end using synthetic sealant and centrifuged in a microhaematocrit centrifuge at 12300 ×g for 5 min. The haematocrit volume was measured using microhaematocrit reader and expressed as percentage of HCT.

Digestive and metabolic enzymology

The digestive enzyme activities were studied in intestine samples of experimental fishes. The α-amylase activity on carbohydrate was estimated by dinitro-salicylic acid method (Rick and Stegbauer, 1974). Protease activity was determined by the casein digestion method (Drapeau, 1976). Lipase activity was determined by the *p*-nitrophenyl palmitate (*p*NPP) hydrolysis method derived from Katsivela et al. (1995).

The aspartate aminotransferase (AST) activity was assayed in liver and muscle tissue homogenates as described by Wootton (1964). The procedure adopted for alanine aminotransferase (ALT) activity was same as for aspartate aminotransferase (AST) activity except the substrate 0.2 M DL- alanine used instead of aspartic acid of ALT procedure. Alkaline phosphatase (ALP) activity was determined by Garen and Levinthal (1960) in liver and intestine tissue homogenates of experimental fish. The acid phosphatase (ACP) activity was estimated using the same method as ALP, except that acetate buffer (0.2M, pH 5) was used in place of bicarbonate buffer. The lactate dehydrogenase (LDH) activity was assayed by the method of Wroblewski and La Due (1955). The malate dehydrogenase (MDH) activity was assayed in

different tissues by the method of Ochoa (1955). The LDH and MDH activity were assayed in liver and muscle tissue homogenates.

Antioxidant defence

Superoxide dismutase was assayed in according to the method described by Misra and Fridovich (1972) based on the oxidation of epinepherine–adrenochrome transition by the enzyme from gill and liver samples. Catalase enzyme activity from gill and liver samples were determined by the method described by Takahara et al. (1960). Gluthathione-S-transferase (GST) activity was determined from gill and liver samples by the method of Habig et al. (1974) using S-2,4-dinitrophenyl glutathione (CDNB) as substrate at 340 nm spectrophotometrically.

Stress biomarkers

The content of liver glycogen was analysed by the anthrone reagent method (Seifter et al., 1950). Blood glucose was estimated by the modified method of Nelson-Somogii (Nelson and Somogyi, 1965). Nitroblue tetrazolium (NBT) assay was done by the method of Stasiack and Baumann (1996) was used for the estimation of respiratory burst activity from blood samples. The acetylcholinesterase activity from the brain samples was assayed by the spectrophotometric method as described by Ellman et al. (1961).

Phagocytic activity

The phagocytic cells were detected with *Staphylococcus aureus* (Bangalore GeNei, India by the method of Anderson - Siwicki (Anderson and Siwicki 1995). One hundred microlitres of blood sample was placed in the wells of microtitre plate followed by 100 µL of *S. aureus* 1×10^7 cells suspended in phosphate buffered saline (pH 7.2) was added and mixed thoroughly. This mixture was incubated for 20 min at room temperature. Then, 5 µL of this mixture was taken on to a clean glass slide and a smear was prepared. The smear was air dried for 5 min. After air drying, fixed with 95% ethanol for 5 min and again air dried. Then the smear was stained with Giemsa stain for 10 min. The phagocytic activity is defined as the number of phagocytising cells (neutrophils) divided by the total number of phagocytes counted. The phagocytic index is expressed as the total number of bacteria engulfed by the phagocytes, divided by the total number of phagocytes containing engulfed bacteria.

Histology

Liver and intestine samples of experimental fishes from respective treatments for histological observations were fixed in Bouin's Fluid. After the fixation, the tissue samples were dehydrated in ethanol series, embedded in paraffin, sectioned at 5µm, mounted on albumin coated glass slides and stained with hematoxylin and eosin (Humason, 1979). The mounted liver sections were examined using the Nikon compound microscope.

Feed economics

The cost of experimental feeds in a commercial scale was calculated from the actual market price of each feed ingredients and additives used in these experimental diets. The feed cost for production of each kg of fish and the relative feed cost savings (in %) of snubnose pompano fed with different experimental diets in comparison with control group was also calculated.

Standardization of cottonseed protein concentrate preparation (CPC)

The protein concentrate preparation protocol from CSM through isoelectric pH precipitation was done by modified Lusas and Rhee (1995) method and standardized for optimum yield of cottonseed protein concentrate. The details of the procedure are as follows: Weigh 100 g of cottonseed meal (CSM) and add 1000 mL of water. Adjust the pH of the slurry to 11 with 10 M NaOH. Stir the mixture for 2 h and monitor the pH at 30 min interval and store at refrigerated condition (4 °C) for overnight. Centrifuge the refrigerated slurry at 7500 rpm for 20 min and collect the supernatant and remove the solid material. Adjust the pH of the supernatant to pH 4.5 (isoelectric) with 6 M HCl to precipitate the protein. Stir the mixture for 30 min and store at refrigerated condition for overnight. Centrifuge the refrigerated solute at 9000 rpm for 20 min to separate the protein and discard the supernatant. The resultant pellet can be lyophilized or dried at 60 °C in the hot air oven to dry as pellet. The dried cottonseed protein concentrate (CPC) was crushed by kitchen mixer grinder to a fine powder of 100 µm particle size and labeled prior to storage in air tight container (Fig. 9). The spent material obtained were also dried and milled to assess its nutritive profile along with cottonseed protein concentrate (Fig. 10)

Fig. 9: Cottonseed protein concentrate (CPC) prepared before and after milling

Fig. 10: The spent material obtained during the process of CPC preparation

Free gossypol isolation

Free gossypol was isolated from cottonseed meal as described by the modified Hron et al. (1996) method. Finely milled cottonseed meal sample was accurately weighed and 3 fold volume of distilled water was added and the slurry was swirled and allowed to stand for 5 min. Seven-fold volume of acetone was added to the slurry and swirled and again allowed to stand for 5 min. The slurry was then filtered through Whatman No. 1 filter paper into another beaker. The filtrate

was concentrated at 50 °C in magnetic stirrer and concentrated until complete removal of water. The pasty gossypol was then stored in refrigerator until used in the experimental feed.

Challenge study and relative percentage of survival

The pathogenic strain of *Vibrio parahaemolyticus* was isolated from the liver of infected snubnose pompano from the wet laboratory by spread plates followed by streak plate to isolate individual colonies. The isolate was identified as *V. parahaemolyticus* by comprehensive biochemical methods (FDA 2004). The culture of *V. parahaemolyticus* was activated by growing it in tryptone soya broth with 1% salt (TCBS) at 37 °C in shaking water bath for 16 h.

After completion of feeding trial and sampling, 8 fishes from each replicate of the same treatments were experimentally infected with pathogenic *Vibrio parahaemolyticus* isolated from infected fish. The pathogenic isolates of *V. parahaemolyticus* were grown in nutrient broth with 1.0% salt at 37 °C for 16 h in a BOD incubator and harvested by centrifuging the culture broth at 4000 rpm for 10 min at 4 °C. The cells were then washed thrice in sterile phosphate buffer saline (PBS) with pH 7.2 and finally maintained in PBS at a dosage of 1.0×10^{7} CFU mL^{-1} used for experimental challenge. The fishes of each treatment group were injected 0.1 mL of this suspension intra-peritoneally. The cumulative mortality (%) patterns were observed in the challenged fishes up to seven days along with the changes in behaviour and morphology. The cause of death was ascertained by re-isolating the bacteria from dead fish liver. The relative percentage of survival (RPS) was calculated as described by Amend (1981).

RPS = Percentage of mortality in treatment group / percentage of mortality in control group ×100

Statistical analysis

The data were statistically analyzed by statistical package, SPSS version 16 (Carver and Nash, 2008). Comparisons among all the treatments were done by one way Analysis of Variance (ANOVA). Comparison between the various parameters of all the treatments was made using Duncan's Multiple Range Test (DMRT) (Duncan, 1955). Comparisons were made at the 5% probability levels and all statements of statistical significance were at $P < 0.05$ or $P<0.001$.

6. RESULTS

Study 1: FM replacement using CSM on growth, feed utilization, digestive and metabolic enzyme activities in the diet of snubnose pompano

Growth and feed utilization

The growth and feed intake of snubnose pompano fed with graded levels of CSM to replace protein content of FM in the diet showed significant difference among the treatments except initial weight. The growth parameters such as body gain (BG), weight gain percentage (WG%), specific growth rate (SGR%), average daily growth (ADG), daily growth co-efficient (DGC), thermal growth co-efficient (TGC) and growth rate on metabolic body weight (GR_{MBW}) were found higher in 0CSM group followed by 100CSM group (Table 5).

The feed utilization parameters such as absolute feed intake (FI_{ABS}), feed intake on % of body weight (FI_{PCT}) and feed intake per metabolic body weight (FI_{MBW}), feed gain ratio (FGR), feed conversion ratio (FCR), protein efficiency ratio (PER), feed efficiency (FE) and protein productive value (PPV) of fish from different treatments showed significant differences ($P<0.001$) and higher values were observed either in 0CSM or 100CSM (Table 6).

Essential amino acid composition of experimental feeds and fishes

Essential amino acid composition of experimental feeds and fishes were showing a significant difference among the feeds except for methionine. The level of arginine and cystine were found higher as the level of CSM increased in the diet (Table 7). There was a slight decrease in the lysine content of the experimental diets among the treatments as the level of inclusion of CSM increased in the diets which is almost on par with the lysine requirement of snubnose pompano. The limiting amino acids, lysine and methionine levels were slightly lower in the 25CSM-100CSM experimental diets fed fishes than the 0CSM diet fed fishes (Table 8). Arginine value was found increasing until 50CSM group after which a slight reduction was noticed in 75CSM and 100CSM groups.

Serum biochemical parameters

The serum biochemical parameters such as serum albumin, globulin and A:G ratio did not show significant difference ($P>0.05$) among the treatments. Serum triglycerides, cholesterol,

phospholipid, very low-density lipoprotein (VLDL) and total lipid contents showed significant difference among the treatments (Table 9). The level of triglycerides, cholesterol, phospholipid, VLDL and total lipid contents were showing a decreasing trend as the level of inclusion of FM was decreased by replaced using CSM and higher values were observed in 0CSM group.

Metabolic enzyme activities

The activities of hepatic metabolic enzyme such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid phosphatase (ACP), alkaline phosphatase (ALP), and malate dehydrogenase (MDH) showed significant difference among the treatments except lactate dehydrogenase (LDH). Higher hepatic ACP, ALP, AST, ALT, LDH and MDH activities were observed in 0CSM group (Table 10). The muscular AST, LDH and MDH enzymes showed significant difference except ALT activity among the treatments (Table 11). The activities were found higher in 0CSM followed by 100CSM group. The intestine ACP and ALP activities showed declining trend as the level of inclusion of CSM in the diet (Fig. 11).

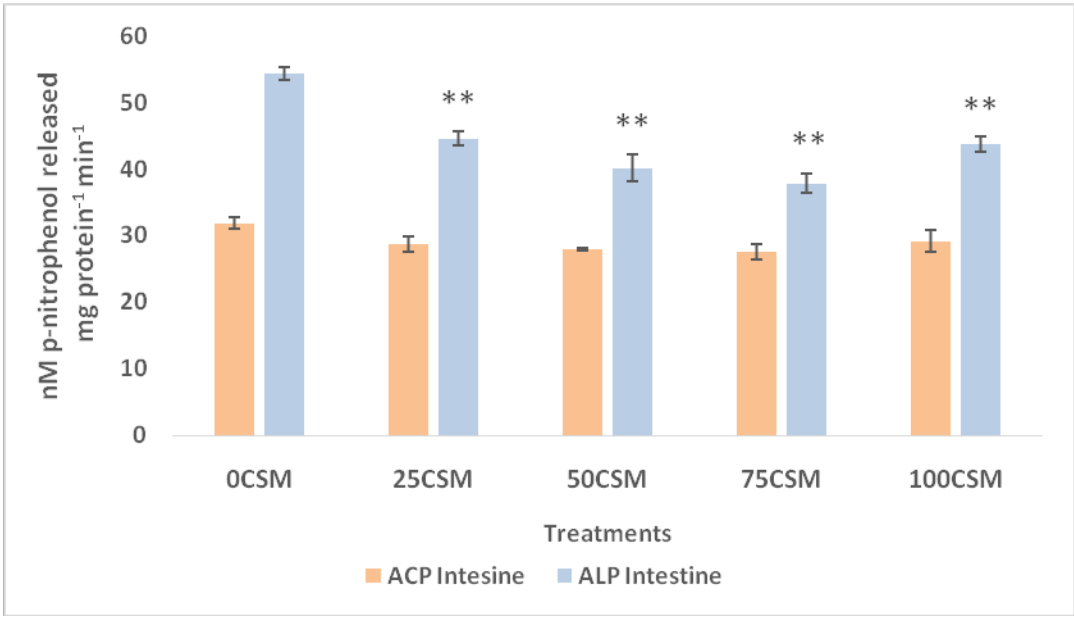


Fig. 11: The ACP and ALP activities of experimental fishes from intestine sample

The level of significance: *significantly different from control at P<0.05 and ** indicates significantly different from control at P<0.001.

Data expressed as arithmetic mean of three replicates ±SE.

F value for intestinal ACP and ALP activities were 2.41and 21.16 respectively; df 4.

Table 5: The growth performance of snubnose pompano fed with graded level of cottonseed meal

Parameters	0CSM	25CSM	50CSM	75CSM	100CSM	df	F value	Level of significance
Initial weight	12.69±0.03	12.75±0.02	12.730.03	12.62±0.05	12.61±0.05	4	2.89	ns
Final weight	61.65^{d}±0.5	56.71^{a}±0.10	57.90^{b}±0.12	57.27^{ab}±0.21	59.06^{c}±0.07	4	57.40	**
BG (g)	48.95^{d}±0.48	43.96^{a}±0.09	45.17^{b}±0.13	44.65^{ab}±0.17	46.45^{c}±0.01	4	65.48	**
WG%	385.68^{d}±3.14	344.81^{a}±1.20	354.72^{b}±1.71	353.78^{b}±1.90	368.30^{c}±1.30	4	75.77	**
SGR%	2.25^{d}±0.10	2.13^{a}±0.03	2.16^{b}±0.06	2.16^{b}±0.03	2.20^{c}±0.04	4	77.70	**
ADG (g day^{-1})	0.70^{d}±0.006	0.63^{a}±0.007	0.65^{b}±0.007	0.64^{ab}±0.05	0.66^{c}±0.006	4	65.48	**
DGC (% day^{-1})	2.31^{d}±0.01	2.15^{a}±0.01	2.19^{b}±0.01	2.18^{b}±0.01	2.23^{c}±0.01	4	65.48	**
W_G (g)	27.97^{c}±0.15	26.89^{a}±0.01	27.15^{ab}±0.03	26.88^{a}±0.10	27.29^{b}±0.06	4	27.22	**
GR_{MBW} (g $kg^{-0.8}$ day^{-1})	31.25^{d}±0.15	29.19^{a}±0.06	29.70^{b}±0.09	29.66^{b}±0.05	30.39^{c}±0.07	4	77.09	**
TGC	0.80^{d}±0.004	0.74^{a}±0.001	0.75^{b}±0.002	0.75^{b}±0.001	0.77^{c}±0.001	4	78.12	**

BG, Biomass gain; WGP, Weight gain percentage; SGR, Specific growth rate; ADG, Average daily growth; W_G, Geometric mean body weight; GR_{MBW}, Growth rate on metabolic body weight; DGC, Daily growth coefficient; TGC, Thermal growth coefficient

Superscripted letters in the same row indicate intergroup statistical differences (Duncan's multiple range test) per level of replacement of FM using CSM: arithmetic means not sharing a common letter are significantly different (P<0·05) and arithmetic means without letters indicate a non significant difference (P>0.05).

The level of significance: ns indicates not significant, * indicates P<0.05 and ** indicates P<0.001.

Data expressed as arithmetic mean of three replicates ±SE.

Table 6: Feed utilization of snubnose pompano fed with graded level of cottonseed meal

Parameters	0CSM	25CSM	50CSM	75CSM	100CSM	df	F value	Level of significance
$FI_{as\ fed}$ (g $fish^{-1}$ day^{-1})	1.12^{d}±0.007	1.03^{a}±0.003	1.05^{b}±0.001	1.06^{bc}±0.003	1.06^{c}±0.002	4	74.97	**
FI_{ABS} (g DM $fish^{-1}$ day^{-1})	1.03^{d}±0.007	0.96^{a}±0.003	0.97^{b}±0.002	0.99^{c}±0.003	0.99^{bc}±0.001	4	53.82	**
FI_{PCT} (% day^{-1})	3.70^{d}±0.008	3.57^{a}±0.008	3.59^{a}±0.005	3.67^{c}±0.004	3.61^{b}±0.009	4	58.85	**
FI_{MBW} (g DM $kg^{-0.8}$ day^{-1})	46.28^{d}±0.11	44.63^{a}±0.11	44.85^{a}±0.07	45.91^{c}±0.05	45.17^{b}±0.11	4	58.85	**
FGR	1.48^{a}±0.006	1.53^{c}±0.004	1.51^{b}±0.002	1.55^{d}±0.001	1.49^{a}±0.002	4	52.72	**
FCR	1.27^{b}±0.01	1.28^{b}±0.02	1.27^{b}±0.02	1.30^{c}±0.03	1.26^{a}±0.02	4	40.37	**
PER	1.56^{d}±0.03	1.52^{b}±0.04	1.53^{c}±0.03	1.50^{a}±0.02	1.56^{d}±0.04	4	42.45	**
FE	0.62^{c}±0.008	0.61^{b}±0.007	0.61^{b}±0.008	0.60^{a}±0.01	0.62^{c}±0.02	4	42.46	**
PPV (%)	34.08^{d}±0.20	33.36^{bc}±0.23	32.82^{ab}±0.27	32.3^{a}±0.17	33.67^{cd}±0.15	4	7.92	**

$FI_{as\ fed}$, Feed intake as fed; FI_{ABS}, Absolute feed intake; FI_{PCT}, Feed intake of fish on % of body weight; FI_{MBW}, Feed intake per on metabolic body weight; FGR, Feed gain ratio on DM basis; FCR, Feed conversion ratio; PER, Protein efficiency ratio; FE, Feed efficiency; PPV, Protein productive value.

Superscripted letters in the same row indicate intergroup statistical differences (Duncan's multiple range test) per level of replacement of FM using CSM: arithmetic means not sharing a common letter are significantly different (P<0·05) and arithmetic means without letters indicate a non significant difference (P>0.05).

The level of significance: ns indicates not significant, * indicates P<0.05 and ** indicates P<0.001.

Data expressed as arithmetic mean of three replicates ±SE.

Table 7: Essential amino acid profile of experimental feeds (g kg^{-1} feed) and fishes fed with graded level of CSM

Amino acids	0CSM	25CSM	50CSM	75CSM	100CSM	Df	F value	Level of significance
Arginine	20.30^{a}±0.08	21.77^{cd}±0.13	22.08^{d}±0.14	21.41^{c}±0.17	20.91^{b}±0.15	4	25.06	**
Histidine	10.15^{e}±0.09	9.70^{d}±0.08	8.80^{c}±0.14	8.19^{b}±0.10	7.66^{a}±0.14	4	83.80	**
Isoleucine	13.39^{d}±0.15	12.75^{c}±0.10	12.41^{bc}±0.11	12.07^{ab}±0.13	11.80^{a}±0.10	4	25.87	**
Leucine	33.06^{d}±0.10	32.48^{c}±0.13	32.07^{b}±0.11	31.73^{a}±0.08	31.44^{a}±0.07	4	29.35	**
Lysine	24.46^{c}±0.07	24.20^{b}±0.04	24.15^{b}±0.03	23.96^{a}±0.02	23.81^{a}±0.07	4	25.34	**
Methionine	11.66±0.01	11.71±0.01	11.71±0.01	11.68±0.01	11.69±0.02	4	3.21	ns
Phenylalanine	17.93^{e}±0.07	16.90^{d}±0.12	16.41^{c}±0.11	15.87^{b}±0.10	15.51^{a}±0.11	4	80.73	**
Therionine	14.96^{e}±0.15	14.41^{d}±0.07	14.06^{c}±0.08	13.56^{b}±0.08	13.22^{a}±0.12	4	42.61	**
Valine	15.04^{d}±0.09	14.70^{c}±0.05	14.20^{b}±0.08	14.07^{ab}±0.080	13.94^{a}±0.05	4	40.13	**
Cystine	6.11^{a}±0.06	6.65^{b}±0.05	7.22^{c}±0.06	8.08^{d}±0.05	8.61^{e}±0.05	4	349.19	**
EAA	165.81^{b}±1.21	163.88^{b}±1.47	161.70^{ab}±1.55	159.62^{a}±1.23	157.78^{a}±0.82	4	6.27	*

EAA, Essential amino acids.

Superscripted letters in the same row indicate intergroup statistical differences (Duncan's multiple range test) per level of replacement of FM using CSM: arithmetic means not sharing a common letter are significantly different (P<0·05) and arithmetic means without letters indicate a non significant difference (P>0.05).

The level of significance: ns indicates not significant, * indicates P<0.05 and ** indicates P<0.001.

Data expressed as arithmetic mean of three replicates ±SE.

Table 8: Essential amino acid profile of experimental fishes (g kg^{-1} body mass on as is basis) fed with graded level of CSM

Amino acids	0CSM	25CSM	50CSM	75CSM	100CSM	Df	F value	Level of significance
Arginine	12.82^{a}±0.01	13.85^{d}±0.04	14.10^{e}±0.05	13.44^{c}±0.06	13.21^{b}±0.02	4	171.79	**
Histidine	8.05^{c}±0.03	7.79^{a}±0.02	8.07^{c}±0.04	8.17^{d}±0.0.03	7.93^{b}±0.02	4	29.52	**
Isoleucine	10.43^{d}±0.06	10.29^{d}±0.04	10.02^{c}±0.05	9.76^{b}±0.03	9.47^{a}±0.09	4	41.50	**
Leucine	17.87^{d}±0.01	17.75^{d}±0.03	16.68^{b}±0.06	16.50^{a}±0.04	16.87^{c}±0.07	4	173.38	**
Lysine	20.69^{b}±0.07	20.36^{ab}±0.04	19.91^{a}±0.03	20.13^{ab}±0.07	19.77^{a}±0.38	4	4.20	*
Methionine	7.10^{c}±0.05	6.95^{b}±0.06	6.88^{ab}±0.03	6.80^{a}±0.02	6.93^{b}±0.03	4	7.24	*
Phenylalanine	8.32^{c}±0.07	7.97^{b}±0.04	7.77^{ab}±0.06	7.92^{ab}±0.03	7.73^{a}±0.12	4	10.65	**
Therionine	7.54^{b}±0.06	7.65^{b}±0.05	7.53^{b}±0.03	7.16^{a}±0.07	6.99^{a}±0.13	4	14.21	**
Valine	10.39^{c}±0.03	10.36^{c}±0.02	10.13^{b}±0.04	10.01^{a}±0.03	10.16^{b}±0.03	4	24.97	**
Cystine	4.54^{ab}±0.05	4.75^{cd}±0.04	4.43^{a}±0.06	4.64^{bc}±0.07	4.84^{d}±0.06	4	8.04	*
EAA	107.76^{c}±0.05	107.30^{c}±0.53	105.29^{b}±0.43	104.53^{ab}±0.11	104.04^{a}±0.25	4	25.96	**

EAA, Essential amino acids.

Superscripted letters in the same row indicate intergroup statistical differences (Duncan's multiple range test) per level of replacement of FM using CSM: arithmetic means not sharing a common letter are significantly different (P<0·05) and arithmetic means without letters indicate a non significant difference (P>0.05).

The level of significance: ns indicates not significant, * indicates P<0.05 and ** indicates P<0.001.

Data expressed as arithmetic mean of three replicates ±SE.

Table 9: Serum biochemical parameters of experimental fishes fed with varying levels of CSM in the diet

Parameters	0CSM	25CSM	50CSM	75CSM	100CSM	df	F value	Level of significance
Protein profile								
Total protein (g dL^{-1})	4.88±0.10	4.72±0.06	4.75±0.11	4.69±0.09	4.83±0.0.09	4	0.77	ns
Albumin (g dL^{-1})	1.97±0.04	1.83±0.04	1.85±0.08	1.81±0.08	1.87±0.04	4	1.04	ns
Globulin (g dL^{-1})	2.91±0.07	2.89±0.02	2.90±0.04	2.87±0.03	2.96±0.05	4	0.53	ns
A:G ratio	0.68±0.01	0.63±0.01	0.64±0.03	0.63±0.03	0.63±0.01	4	1.11	ns
Lipid profile								
Triglyceride (mg dL^{-1})	122.62^{b}±2.83	116.07^{a}±1.79	111.01^{a}±2.59	115.18^{a}±1.03	111.31^{a}±2.08	4	5.20	*
Cholesterol (mg dL^{-1})	150.81^{b}±1.01	147.57^{ab}±1.83	146.15^{a}±1.23	144.47^{a}±1.36	143.56^{a}±1.12	4	4.50	*
Phospholipids (mg dL^{-1})	200.09^{b}±0.73	197.73^{ab}±1.34	196.69^{a}±0.90	195.46^{a}±1.00	194.80^{a}±0.82	4	4.51	*
Total lipid (mg dL^{-1})	473.52^{c}±2.46	461.37^{b}±2.60	453.85^{ab}±2.06	455.10^{ab}±2.50	449.67^{a}±2.07	4	15.64	**
VLDL (mg dL^{-1})	24.52^{b}±0.57	23.21^{a}±0.21	22.20^{a}±0.52	23.04^{a}±0.21	22.26^{a}±0.42	4	5.20	*

VLDL, Very low density lipoprotein.

Superscripted letters in the same row indicate intergroup statistical differences (Duncan's multiple range test) per level of replacement of FM using CSM: arithmetic means not sharing a common letter are significantly different (P<0·05) and arithmetic means without letters indicate a non significant difference (P>0.05).

The level of significance: ns indicates not significant, * indicates P<0.05 and ** indicates P<0.001. Data expressed as arithmetic mean of three replicates ±SE.

Table 10: Metabolic enzyme activities in the liver tissue of experimental fishes fed with varying levels of CSM in the diet

Parameter	0CSM	25CSM	50CSM	75CSM	100CSM	df	F value	Level of significance
ACP	70.04^{b}±2.10	62.42^{ab}±3.40	59.42^{a}±4.22	54.23^{a}±1.67	59.89^{a}±0.78	4	4.47	*
ALP	108.83^{c}±3.75	99.29^{b}±0.66	95.81^{b}±2.05	88.05^{a}±1.08	94.65^{ab}±1.82	4	12.47	**
AST	31.85^{c} ±1.63	27.81^{b} ±0.88	25.04^{b} ±1.81	26.61^{a} ±0.24	27.32^{b} ±0.38	4	4.62	*
ALT	24.63^{c}±0.26	20.80^{c}±0.66	21.23^{b} ±0.61	22.34^{a}±1.34	22.13^{c}±0.38	4	3.94	*
LDH	0.99±0.03	0.83±0.04	0.85±0.09	0.90±0.03	0.89±0.05	4	1.43	ns
MDH	2.78^{c} ±0.04	2.61^{abc}±0.07	2.46^{a}±0.07	2.53^{ab}±0.08	2.70^{bc}±0.06	4	4.14	*

ALT, Alaninae aminotransferase; AST, Aspartate aminotransferase; LDH, Lactate dehydrogenase; MDH, Malate dehydrogenase.

LDH and MDH activity was expressed as Unit mg protein^{-1} min^{-1} where 1 unit was equal to A0.01 OD min^{-1}. AST and ALT activity was expressed as nanomoles oxaloacetate formed mg protein^{-1} min^{-1} and nanomoles pyruvate formed mg protein^{-1} min^{-1} respectively. ACP and ALP activity was expressed as nanomoles p-nitrophenol released min^{-1} mg protein^{-1}

Superscripted letters in the same row indicate intergroup statistical differences (Duncan's multiple range test) per level of replacement of FM using CSM: arithmetic means not sharing a common letter are significantly different (P<0·05) and arithmetic means without letters indicate a non significant difference (P>0.05).

The level of significance: ns indicates not significant, * indicates P<0.05 and ** indicates P<0.001.
Data expressed as arithmetic mean of three replicates ±SE.

Table 11: Metabolic enzyme activities in the muscle tissues of experimental fishes fed with varying levels of CSM in the diet

Parameter	0CSM	25CSM	50CSM	75CSM	100CSM	df	F value	Level of significance
AST	17.62^{d} ±0.29	16.51^{c} ±0.43	15.97^{ab}±0.24	15.34^{a} ±0.54	17.02^{bc} ±0.22	4	5.88	*
ALT	11.83±0.29	10.62±0.54	9.83±0.97	10.20±0.75	11.07±0.2	4	1.58	ns
LDH	1.40^{c}±0.02	1.24^{ab}±0.04	1.14^{a}±0.03	1.19^{ab}±0.02	1.28^{b} ±0.05	4	8.34	*
MDH	3.78^{c}±0.05	3.61^{b}±0.03	3.51^{b}±0.03	3.35^{a}±0.06	3.74^{c}±0.02	4	22.81	**

ALT, Alaninae aminotransferase; AST, Aspartate aminotransferase; LDH, Lactate dehydrogenase; MDH, Malate dehydrogenase.

LDH and MDH activity was expressed as Unit mg $protein^{-1}$ min^{-1} where 1 unit was equal to A0.01 OD min^{-1}. AST and ALT activity was expressed as nanomoles oxaloacetate formed mg $protein^{-1}$ min^{-1} and nanomoles pyruvate formed mg $protein^{-1}$ min^{-1} respectively.

Superscripted letters in the same row indicate intergroup statistical differences (Duncan's multiple range test) per level of replacement of FM using CSM: means not sharing a common letter are significantly different ($P<0.05$) and mean without letters indicate a non significant difference ($P>0.05$).

The level of significance: ns indicates not significant, * indicates $P<0.05$ and ** indicates $P<0.001$.
Data expressed as arithmetic mean of three replicates ±SE

Feed economics

The cost of experimental feeds was in the range of Rs.70.35-78.24 (Table 12). The feed cost decreased considerably as the level of inclusion of CSM increased in the diet in place of FM. The relative percentage of feed cost in comparison with 0CSM diet was lowered in the range of 2.96-10.08 % as the inclusion of CSM increased in the diet (Fig. 12A). Feed cost per kilogram of fish production was found lowest (Rs. 88.64) in 100CSM group than the other groups and maximum cost (Rs.99.36) was registered in 0CSM group (Fig. 12B).

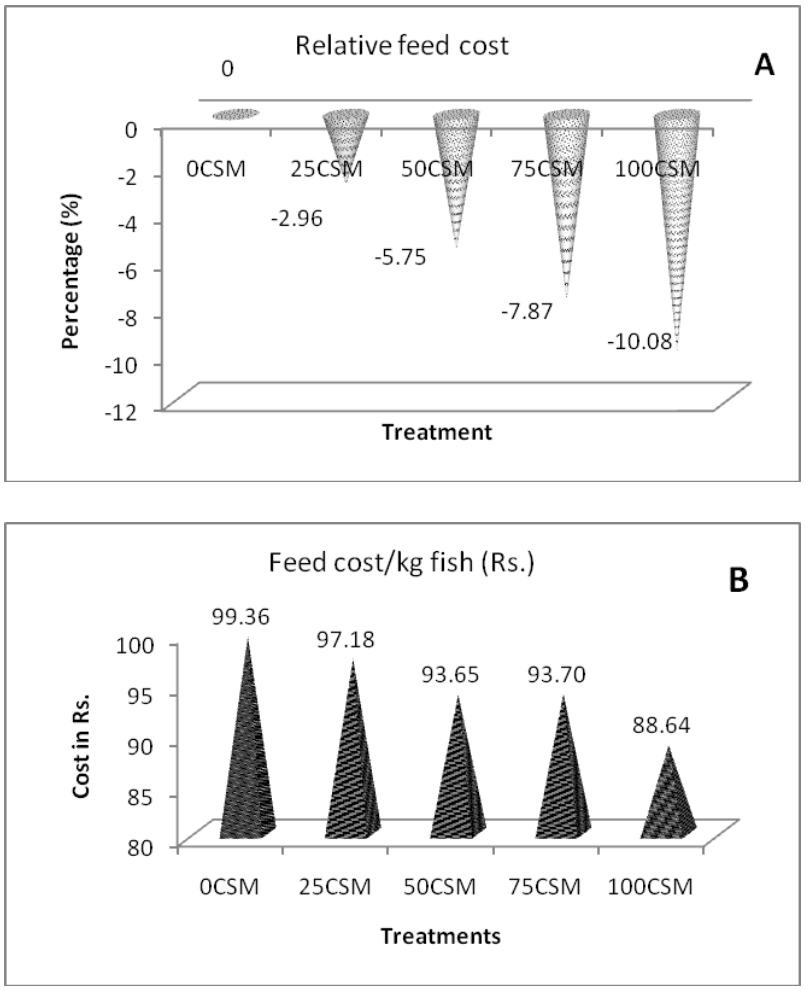


Fig. 12. Feed economics in snubnose pompano fed with varying level of dietary CSM

A. The relative feed cost (in %) of different experimental diets in comparision with the control diet (0CSM); B. Feed cost (in Rs.) for each kg of snubnose pompano production in different treatments.

Table 12: Feed economics of experimental diets

Ingredients	Price/ kg	Price in Rs.				
	(Rs.) †	0CSM	25CSM	50CSM	75CSM	100CSM
Cottonseed meal	40.0	0.000	3.045	6.090	9.100	12.145
Soybean meal	33.0	4.620	4.950	5.280	5.577	5.808
Squid meal	90.0	3.600	3.600	3.600	3.600	3.600
Meat and bone meal	35.0	1.750	1.750	1.750	1.750	1.750
Groundnut oil cake	45.0	5.670	5.805	5.738	5.805	5.805
Shrimp meal	40.0	2.000	2.000	2.000	2.000	2.000
Fishmeal	83.0	19.920	14.940	9.960	4.980	0.000
Wheat powder	24.0	4.800	4.560	4.320	3.720	2.820
De-oiled rice bran	22.0	1.606	0.935	0.330	0.000	0.000
Sunflower oil	70.0	0.700	0.700	0.700	0.700	0.700
Fish oil	100.0	1.500	1.500	1.500	1.500	1.500
Vitamin mixture	350.0	5.250	5.250	5.250	5.250	5.250
Mineral mixture	60.0	0.900	0.900	0.900	0.900	0.900
Vitamin C	1200.0	3.000	3.000	3.000	3.000	3.000
Dicalcium phosphate	150.0	0.750	0.750	0.750	0.750	0.750
DL-Methionine*	300.0	1.050	1.050	1.200	1.350	1.500
Lysine*	125.0	0.375	0.438	0.625	0.750	0.875
Immunostimulant	250.0	1.250	1.250	1.250	1.250	1.250
Lecithin	250.0	1.250	1.250	1.250	1.250	1.250
Binder	300.0	0.000	0.000	0.000	0.600	1.200
BHT*	200.0	0.200	0.200	0.200	0.200	0.200
SMB*	250.0	0.250	0.250	0.250	0.250	0.250
Total cost of feed formula (Rs kg^{-1})		60.441	58.123	55.943	54.282	52.553
Manufacturing cost (Rs kg^{-1}) **		17.8	17.8	17.8	17.8	17.8
Total cost (Rs kg^{-1})		78.24	75.92	73.74	72.08	70.35

*indicates commercial grade price

**includes: Production cost, bagging cost, manufacturer &dealer margin & Transportation cost;

† indicates the price of ingredients in India during the month of May 2021

BHT, Butylated hydroxyl toluene; SMB, Sodium metabisulfite

Free gossypol content

As muscle tissue is edible, in the current study FG content from muscle tissue was assessed. The FG content of muscle samples among the treatments showed significant difference ($P<0.05$). The higher FG content of 3.95 mg kg^{-1} on dry matter basis was observed in 100CSM group. The level of FG contents of CSM based diet fed fishes were very well below the limit set by FDA (450 mg kg^{-1}). The relationship between muscular FG accretion (mg kg^{-1}) on dry matter basis and dietary FG content showing a linear equation of $y = 0.0115x + 0.1712$. $R^2 = 0.9892$ (Fig. 13).

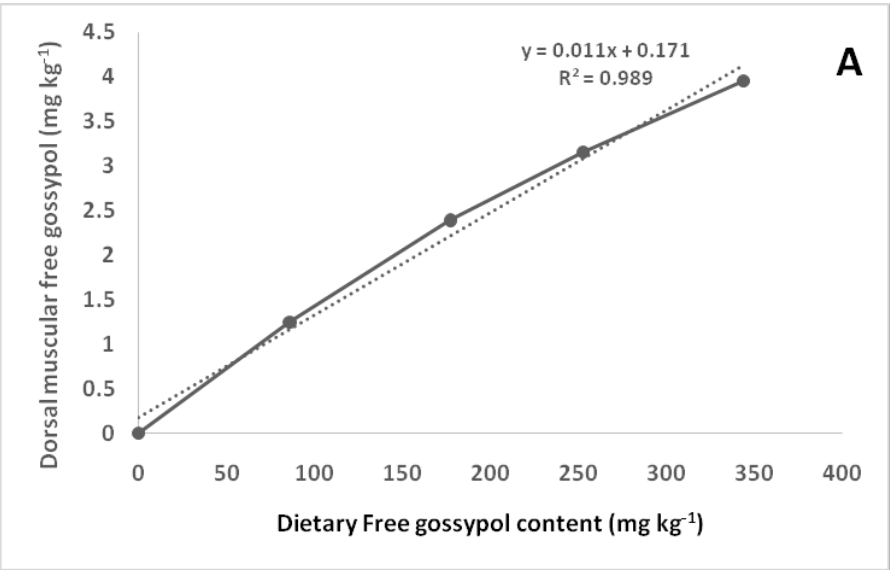


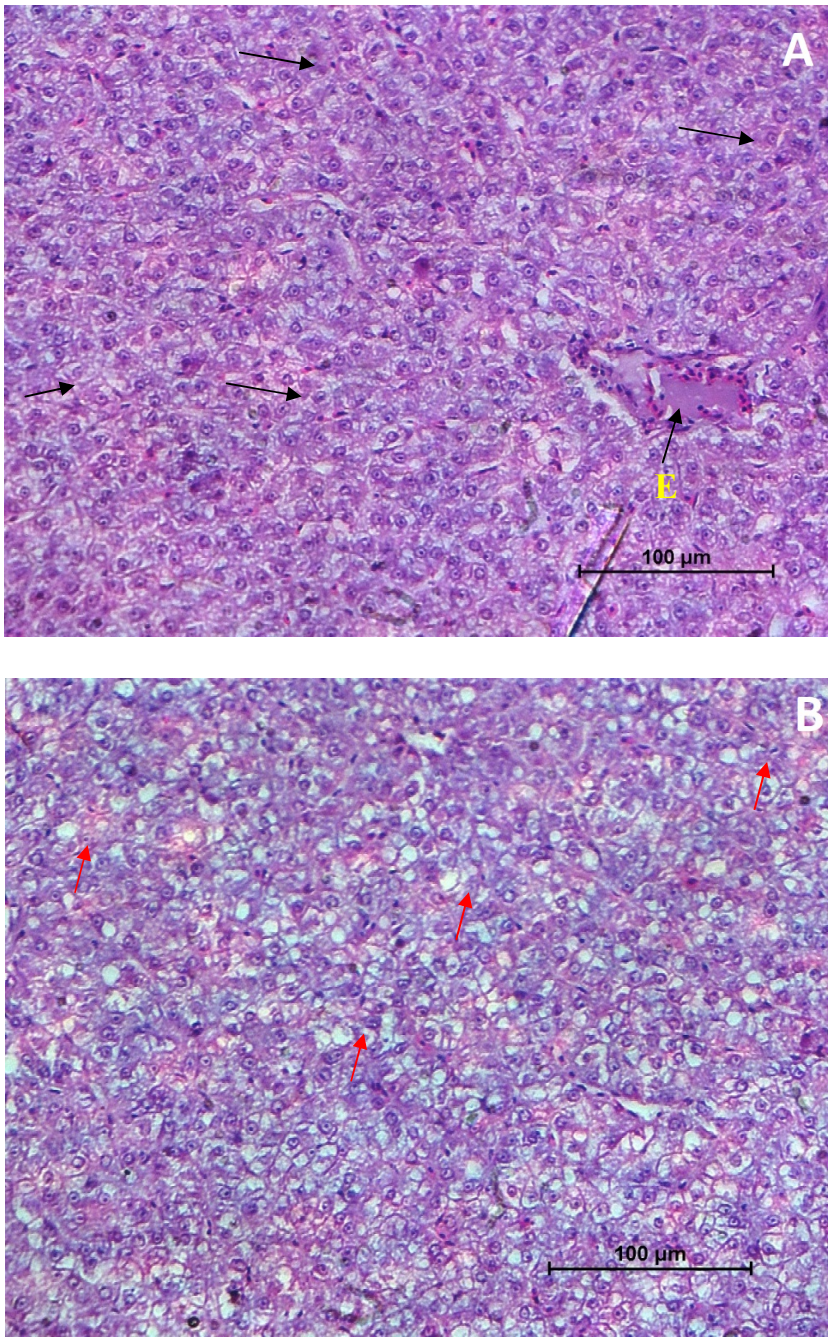
Fig. 13: Free gossypol content in the muscle tissues of experimental fishes

The linear regression relationship between dietary FG content and muscular FG accretion (DM basis) in the experimental fishes fed with varying levels of CSM incorporated diet.

Data expressed as arithmetic mean of three replicates ± SE.

Liver histology of experimental fishes

Liver sections of snubnose pompano fed with various levels of CSM are depicted (Fig. 14). Liver section of snubnose pompano from 25CSM group showing the hepatocytes with mild intra cytoplasmic vacuolation and also in 50CSM group very few mild vacuolated hepatocytes, some hepatocytes with pyknotic nucleus and very few hepatocytes with hypertrophy were present. The hepatocytes of liver section of 100CSM group were normal with no abnormalities and were comparable with 0CSM.


A
E
100 µm
B
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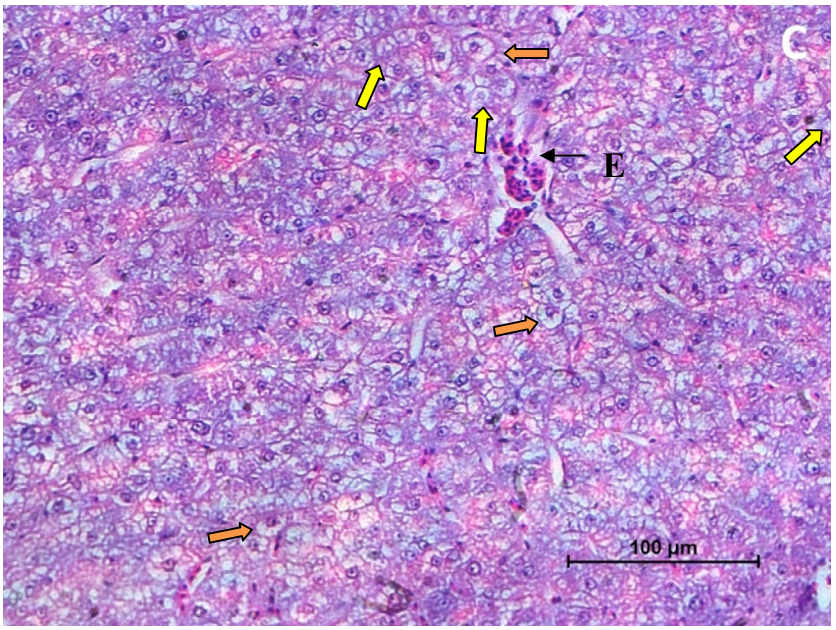
C
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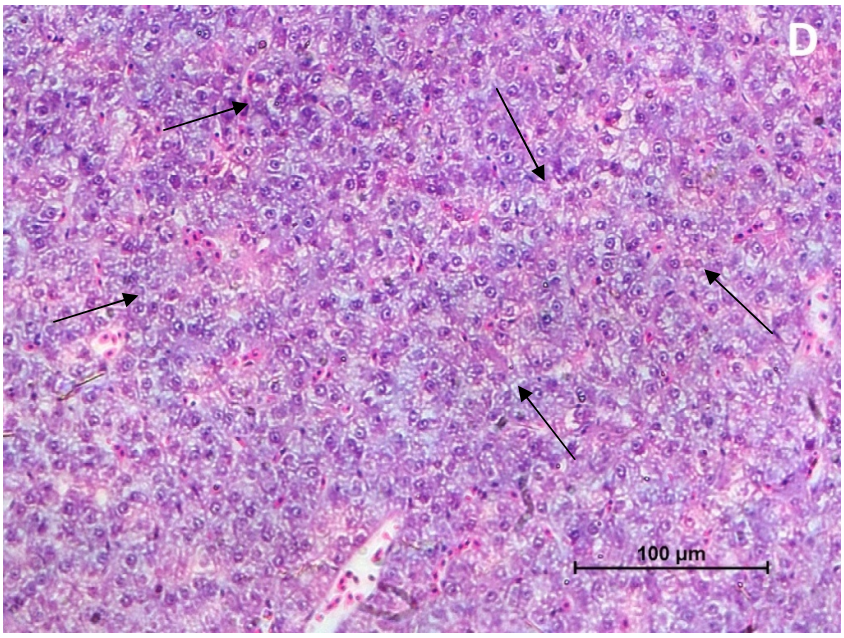

D
100 µm

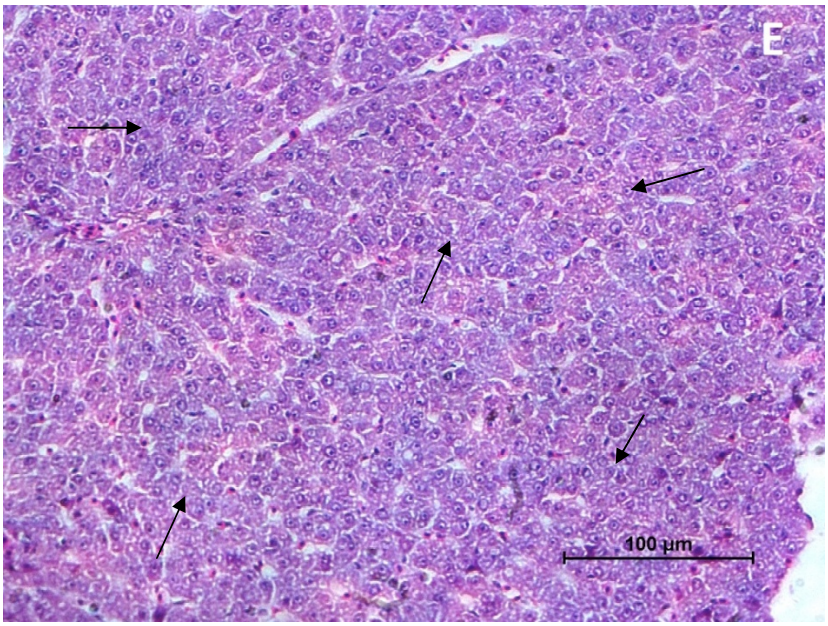


Fig. 14: Liver histology of experimental fishes fed with graded level of CSM to replace fish meal in the diet (stained with H&E; X20).

A. Liver section of snubnose pompano from 0CSM group showing the healthy hepatocytes with normal nucleus (black arrow). The erythrocytes (E) found in the blood vessels are normal. B. Liver section of snubnose pompano from 25CSM group showing the hepatocytes with mild intra cytoplasmic vacuolatin (red arrow); C. Liver section of snubnose pompano of 50CSM group showing the hepatoctes with few pyknotic nucleus (yellow arrow) and very few hepatocytes with hypertrophy (Orange arrow) are also present. The erythrocytes found in the blood vessels are normal; D. Liver section of snubnose pompano of 75CSM group showing normal healthy hepatocytes with normal nucleus (black arrow); E. The hepatocytes of liver section of 100CSM group are normal and no abnormalities are seen (black arrow).

Study 2:

Preparation of cottonseed protein concentrate (CPC) and its effect in snubnose pompano by replacing FM

The method of cottonseed protein concentrate (CPC) preparation through isoelectric precipitation was standardized and the protocol witnessed the yield of 28.35±3.44% (Mean ±SE) of CPC from the CSM processed. The nutritive profile in terms of crude protein content was assessed and the protein content of CPC was 69.47% (Table 13). The protein content of

CSM and spent (SP) materials were 42.11 and 21.53%, respectively. The gossypol content showed a reduction of 79.73 % in CPC than CSM.

Table 13: Proximate composition analysis of cotton seed products and fishmeal (% as fed)

Parameter	CSM	CPC	SP	FM
Moisture	9.71±0.52	7.77±0.41	6.85±0.17	6.76±0.29
Crude protein	42.11±0.29	69.47±1.28	1.19±0.20	63.34±0.27
Crude lipid	3.97±0.12	0.63±0.04	4.95±0.09	7.08±0.27
Total ash	7.46±0.12	2.93±0.05	10.79±0.31	19.87±0.13
Crude fibre	4.65±0.14	0.34±0.03	7.78±0.08	0.23±0.01
NFE	32.10±0.47	18.85±0.85	48.42±0.60	2.68±0.28
Gross energy (kcal kg^{-1})	3959.1±9.23	4583.3±33.25	3590.3±17.50	4189.9±8.01
Free gossypol (mg kg^{-1})	965.67±11.56	195.68±5.23	732.33±9.33	0.00

CSM, Cotton seed meal; CPC, Cotton seed protein concentrate; SP, Spent; FM, fishmeal: NFE, Nitrogen free extract.

The data is represented as arithmetic mean of three replicates ±SE.

Amino acid profile of CPC

The amino acid profile of CPC, CSM and SP were analysed and represented along with FM in table 14. The limiting amino acids, lysine and methionine levels of CPC showed an increase of 31.8 and 54.39% respectively than CSM.

Table 14: Amino acid profile analysis cotton seed products and fishmeal (g kg^{-1})

Parameter	CSM	CPC	SP	FM
Essential amino acid (EAA)(g kg^{-1})				
Arginine	36.26±0.76	58.45±0.84	12.23±0.65	32.26±0.95
Histidine	9.45±0.60	15.84±0.82	4.17±0.34	12.55±0.70
Lysine	15.44±0.99	20.35±1.21	3.89±0.16	49.85±1.95
Isoleucine	12.63±0.64	19.48±0.97	5.82±0.24	24.55±1.21
Leucine	21.11±0.87	34.19±1.56	6.92±0.25	44.76±2.18
Methionine	5.92±0.19	9.14±0.37	2.12±0.10	16.33±0.90
Phenylalanine	20.44±0.98	29.56±0.91	5.10±0.27	27.57±2.08
Threonine	12.57±0.66	19.27±0.64	3.30±0.09	29.45±1.20
Tyrosine	8.75±0.40	14.37±0.69	3.56±0.14	18.95±0.89

Valine	17.22±0.84	24.44±0.64	7.82±0.36	27.65±1.13
Cystine	6.58±0.25	8.74±0.28	2.12±0.12	5.34±0.14
∑EAA	166.37±4.28	253.83±4.68	57.05±2.31	289.26±10.88
Non-essential amino acid (NEAA)(g kg^{-1})				
Alanine	8.95±0.44	15.56±0.71	4.67±0.13	16.45±0.90
Glycine	13.45±0.65	19.80±0.89	7.80±0.32	26.90±1.41
Glutamine	41.85±1.85	62.31±2.87	6.89±0.31	69.78±3.02
Glutamic acid	9.10±0.40	14.45±0.64	3.23±0.13	22.65±1.22
Asparagine	16.10±0.77	24.43±1.23	4.57±0.25	33.45±1.74
Aspartic acid	21.46±1.03	35.89±1.81	5.29±0.16	38.78±1.54
Hydroxyproline	10.20±0.57	16.78±0.71	3.82±0.12	23.32±0.89
Proline	22.11±1.21	36.78±1.28	5.40±0.16	43.56±2.37
Serine	15.60±0.63	23.31±1.16	5.10±0.23	17.80±0.71
∑NEAA	158.82±3.86	249.31±9.89	46.77±1.86	292.69±13.82

CSM, Cotton seed meal; CPC, Cotton seed protein concentrate; SP, Spent; FM, fishmeal: Data expressed as arithmetic mean of three replicates ±SE

Growth response and feed utilization

The growth performance of snubnose pompano fed with graded levels of CPC to replace protein content of FM in the diet showed significant difference ($P<0.05$) in weight gain percentage, SGR, TGC and DGC (Table 15). The FCR, FE, PER, PPV (%), ERE (%) and NRE (%) showed a significant difference ($P<0.05$) among the treatments (Table 16).

Proximate composition of experimental fishes

The proximate composition of experimental fishes is given in Table 17. The moisture, crude protein, total ash and calcium content of experimental fishes showed significant difference ($P<0.05$) and total lipid, NFE and phosphorous content did not show significant difference ($P>0.05$). The crude protein, total ash and calcium content of whole-body samples exhibited a decreasing trend as the level of inclusion of CPC increased in the diet.

Table 15: The growth response of pompano fed with varying levels of CPC in the diet

Parameter	0CPC	25CPC	50CPC	75CPC	100CPC	df	F value	Level of significance
Initial weight (g)	3.96±0.21	4.04±0.04	4.09±0.10	4.21±0.08	4.17±0.11	4	0.657	ns
Final weight (g)	15.48±0.97	15.50±0.19	15.51±0.54	15.33±0.17	14.69±0.29	4	0.442	ns
BG(g)	11.52±0.77	11.46±0.17	11.43±0.43	11.13±0.14	10.53±0.22	4	0.979	ns
WGP (%)	290.57^{c}±3.90	283.53^{c}±3.71	279.82^{bc}±2.98	264.56^{ab}±6.21	252.78^{a}±7.00	4	9.343	**
SGR (%)	1.94^{c}±0.01	1.92^{c}±0.02	1.91^{bc}±0.01	1.85^{ab}±0.02	1.80^{a}±0.03	4	8.967	**
ADG (g)	0.164±0.01	0.164±0.002	0.163±0.006	0.159±0.002	0.150±0.003	4	0.982	ns
DGC	1.30^{b}±0.03	1.29^{b}±0.01	1.28^{b}±0.02	1.24^{ab}±0.01	1.20^{a}±0.02	4	3.568	*
TGC	0.448^{b}±0.01	0.443^{b}±0.003	0.441^{b}±0.007	0.429^{ab}±0.005	0.414^{a}±0.006	4	3.568	*
Survival (%)	87.50^{ab}±7.21	100.00^{b}±0.00	87.50^{ab}±7.21	83.33^{ab}±8.33	66.67^{a}±4.17	4	3.773	*

BG, Biomass gain; WGP, Weight gain percentage; SGR, Specific growth rate; ADG, Average daily growth; DGC, Daily growth co-efficient; TGC, Thermal growth coefficient.

Superscripted letters in the same row indicate intergroup statistical differences (Duncan's multiple range test) per level of replacement of FM using CPC: arithmetic means not sharing a common letter are significantly different (P<0·05) and arithmetic means without letters indicate a non significant difference (P>0.05).

The level of significance: ns indicates not significant, * indicates P<0.05 and ** indicates P<0.001.

Data expressed as arithmetic mean of three replicates ±SE

Table 16: The feed utilization of pompano fed with varying levels of CPC in the diet

Parameter	0CPC	25CPC	50CPC	75CPC	100CPC	df	F value	Level of significance
FI (g)	21.75±0.85	21.04±0.20	21.25±0.36	21.71±0.22	21.88±0.33	4	0.611	ns
FCR	1.90 ^{a}±0.06	1.84 ^{a}±0.04	1.86 ^{a}±0.07	1.95 ^{ab}±0.04	2.08 ^{b}±0.02	4	3.982	*
FE	0.526 ^{b}±0.02	0.543 ^{b}±0.01	0.536 ^{b}±0.02	0.513 ^{ab}±0.01	0.477 ^{a}±0.003	4	3.645	*
PER	0.758 ^{a}±0.02	0.735 ^{a}±0.02	0.745 ^{a}±0.03	0.781 ^{ab}±0.02	0.832 ^{b}±0.006	4	4.020	*
PPV (%)	27.65^{b}±0.75	28.38^{b}±0.60	27.83^{b}±1.02	26.30^{ab}±0.59	24.80^{a}±0.19	4	4.511	*
ERE (%)	22.16^{b}±0.62	22.75^{b}±0.41	22.34^{b}±0.84	21.07^{ab}±0.49	19.92^{a}±0.14	4	4.348	*
NRE (%)	30.72^{b}±0.84	31.54^{b}±0.63	30.93^{b}±1.13	29.23^{ab}±0.65	27.55^{a}±0.22	4	4.511	*

FI, Feed intake; FCR, Feed conversion ration; FE, Feed efficiency; PER, Protein efficiency ratio; PPV, Protein productive value; ERE, Energy retention efficiency; NRE, Nitrogen retention efficiency.

Superscripted letters in the same row indicate intergroup statistical differences (Duncan's multiple range test) per level of replacement of FM using CPC: arithmetic means not sharing a common letter are significantly different (P<0·05) and arithmetic means without letters indicate a non significant difference (P>0.05).

The level of significance: ns indicates not significant, * indicates P<0.05 and ** indicates P<0.001.

Data expressed as arithmetic mean of three replicates ±SE

Table 17: The proximate composition of whole-body tissues of experimental fishes (% as fed)

Parameters	0CPC	25CPC	50CPC	75CPC	100CPC	df	F value	Level of significance
Moisture	68.65^{a}±0.04	68.76^{a}±0.04	68.94^{b}±0.06	69.16^{bc}±0.07	69.09^{c}±0.07	4	13.33	**
OM	26.41^{d}±0.04	26.39^{cd}±0.05	26.22^{bc}±0.08	26.02^{a}±0.05	26.12^{ab}±0.05	4	9.08	**
Crude protein	20.19^{c}±0.04	20.11^{bc}±0.04	20.00^{ab}±0.07	19.84^{a}±0.04	19.89^{a}±0.03	4	9.50	**
Crude lipid	6.09±0.01	6.11±0.02	6.07±0.01	6.01±0.02	6.06±0.03	4	3.46	ns
Total ash	4.92^{b}±0.02	4.85^{a}±0.02	4.83^{a}±0.03	4.82^{a}±0.02	4.80^{a}±0.03	4	4.21	*
Crude fibre	0.065^{a}±0.009	0.088^{b}±0.002	0.083^{b}±0.002	0.081^{b}±0.002	0.082^{b}±0.002	4	4.26	*
NFE	0.078±0.007	0.083±0.007	0.075±0.002	0.082±0.008	0.080±0.009	4	0.23	ns
GE (kcal kg^{-1})	1641^{d}±2.81	1693^{cd}±2.98	1629^{bc}±4.89	1616^{a}±3.76	1623^{ab}±3.05	4	8.86	**
Calcium	5.00^{c}±0.06	4.95^{c}±0.08	4.76^{b}±0.04	4.70^{ab}±0.04	4.56^{a}±0.06	4	10.01	**
Phosphorous	2.87±0.09	2.89±0.07	2.75±0.02	2.70±0.02	2.78±0.02	4	2.41	ns

OM, Organic matter; NFE, Nitrogen free extract; GE, Gross energy.

Superscripted letters in the same row indicate intergroup statistical differences (Duncan's multiple range test) per level of replacement of FM using CSM: arithmetic means not sharing a common letter are significantly different (P<0·05) and arithmetic means without letters indicate a non significant difference (P>0.05).

The level of significance: ns indicates not significant, * indicates P<0.05 and ** indicates P<0.001.

Data expressed as arithmetic mean of three replicates ±SE.

Digestive enzyme activities

The intestinal protease activity showed significant difference (P<0.001) among the treatments (Fig.15). Among the treatments higher protease activity was witnessed in 0CPC group and the activity was decreased as the level of inclusion of CPC increased in the diet. There was no significant difference (P>0.05) in the α-amylase and lipase activities among the treatments.

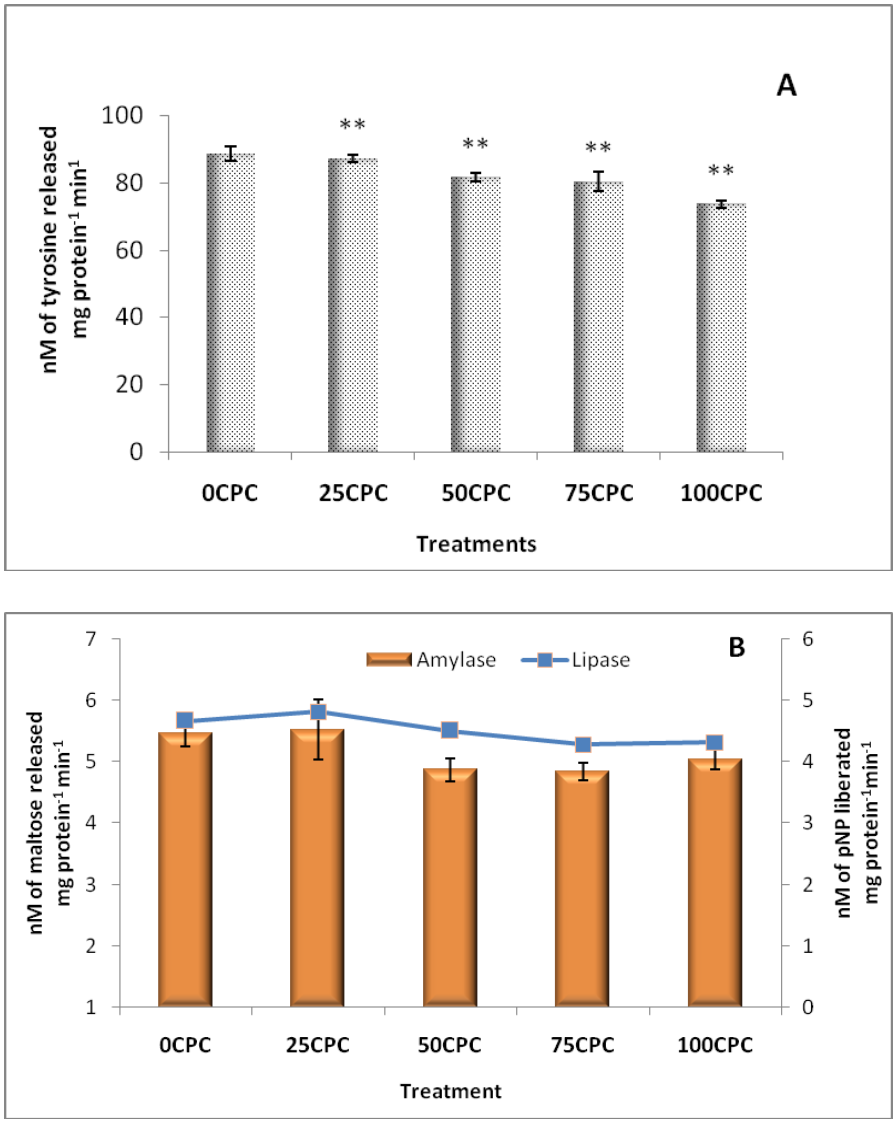
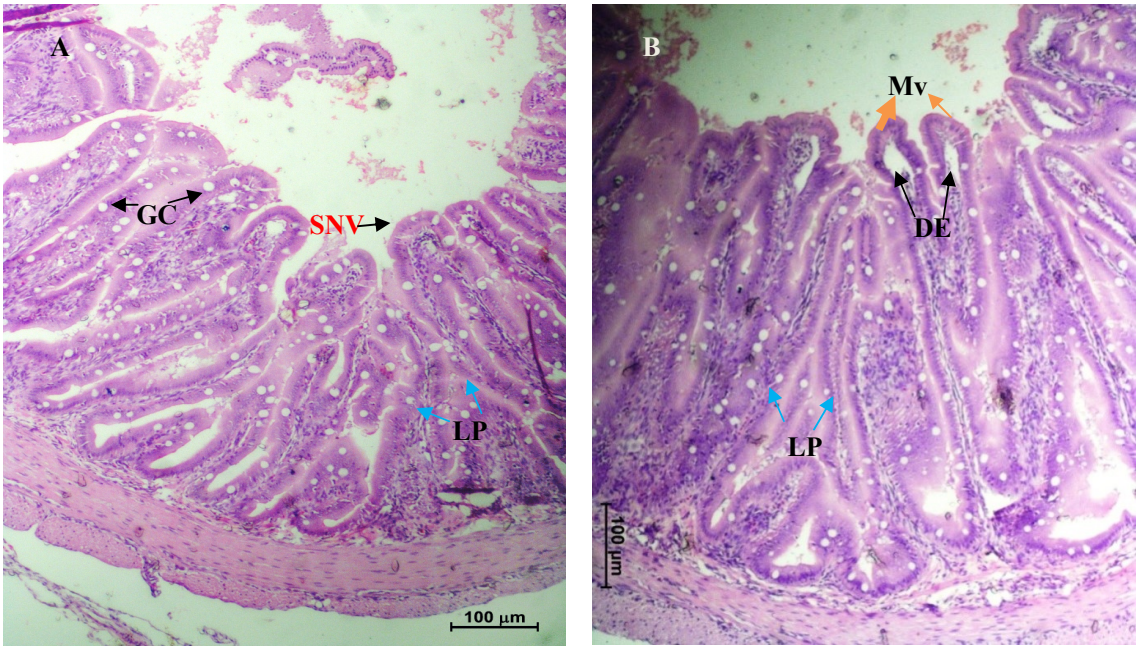


Fig. 15: The intestinal digestive enzyme activities (A. Protease; B. α-amylase and lipase) of snubnose pompano fed with various levels of CPC in the diet

The level of significance: *significantly different from control at P<0.05 and ** indicates significantly different from control at P<0.001. Data expressed as arithmetic mean of three replicates ±SE. F value for intestinal protease activity is 11.45; df 4.

Intestinal morphology

In the present study, the liver and intestinal morphology was assessed to understand the effect of CPC in snubnose pompano as they are the very essential organ in digestion and metabolism nutrients in the diet. In the present study, the reduction in the number of lengthwise aligned absorptive cells with supranuclear vacuolisation (SNV) of the enterocytes in the mucosal folds (MF) was seen in higher inclusion of CPC in the diet. The thickness of lamina propria (LP) has increased with a mild infiltration of inflammatory cells (IC) especially neutrophils and size of goblet cells (GC) slightly increased and not the numbers in the epithelium of fishes fed with higher inclusion of CPC (75CPC, 100CPC) in the diet (Fig. 16). The branching or undulations of mucosal folds (MF) are noticed as the level of inclusion of CPC increased in the diet. Necrotic lesions (N) observed in the microvilli (Mv) seen by detachment of surface epithelial cells from underlying lamina propria of fishes fed with diet containing complete replacement of fishmeal using CPC (100CPC).



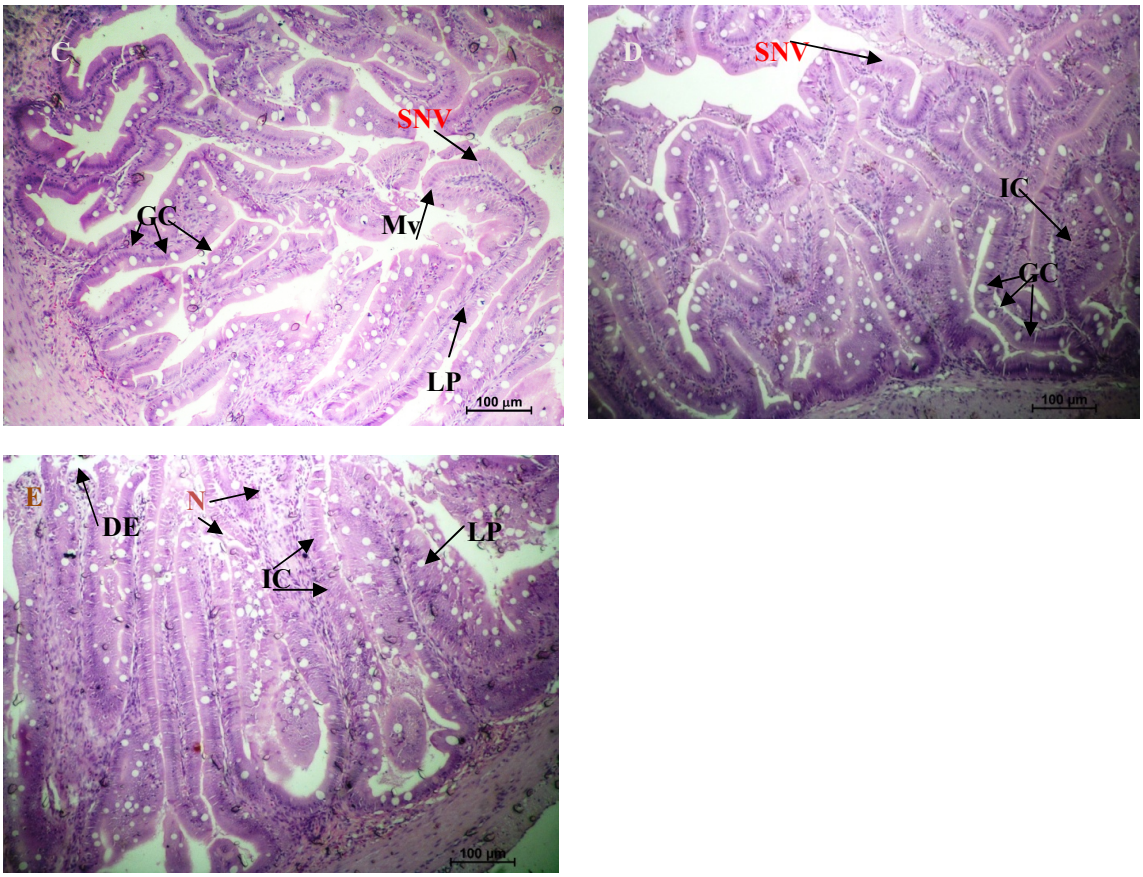
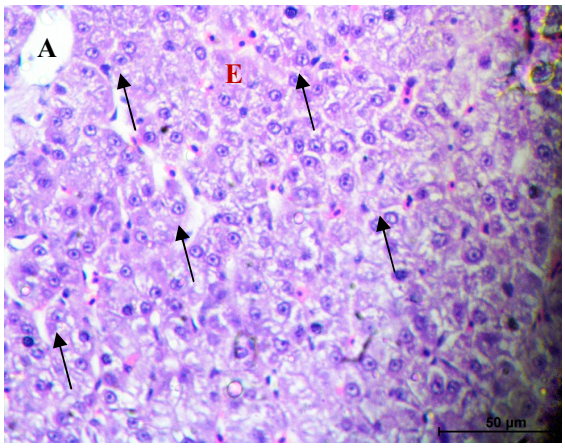


Fig. 16: The distal intestine section of snubnose pompano fed with various levels of CPC to replace fishmeal in the diet A) 0CPC; B. 25CPC; C. 50CPC; D. 75CPC and E. 100CPC treatments.

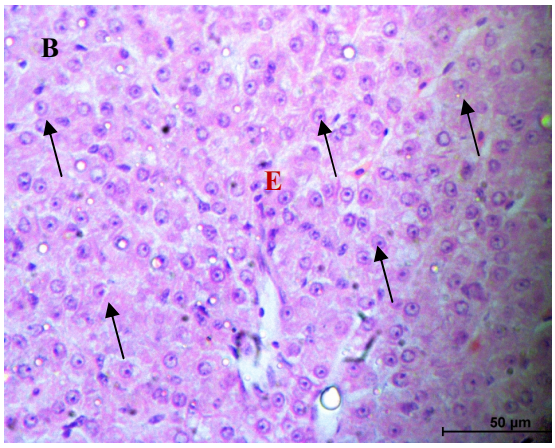
SNV, supranuclear vacuolization; LP, lamina propria; IC, inflammatory cells; MF, mucosal folds; N, Necrotic lesions; Mv, microvilli; GC, goblet cells.

Liver histology of snubnose pompano fed with CPC

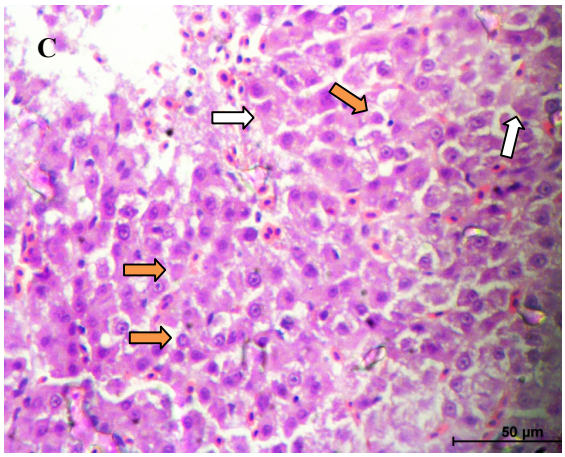
Liver sections of snubnose pompano from various treatments indicates that structural changes as the inclusion of CPC increased in the diet (Fig. 17). Liver section of snubnose pompano from 0CPC and 25CPC group was showing the normal hepatocytes with apparently no signs of damage. The liver sections of 50CPC group showed mostly normal hepatocytes and some hepatocytes with pyknotic nucleus and the blood capillaries seen with mild congestion of erythrocytes. The hepatocytes of liver section of 75CPC group were seen with mild vacculation of hepatocytes and pyknotic nucleus and also tissue lesions and damage of connective tissues were also noticed. Liver section of 100CPC group fish showed mostly cytoplasmic round circumscribed vacuolation of hepatocytes along with increased number of erythrocytes.



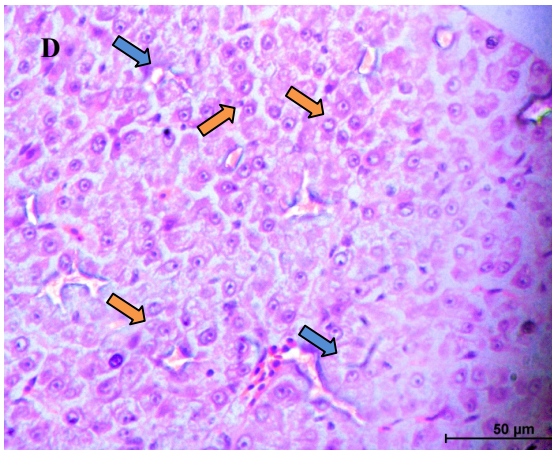
Liver section of snubnose pompano fed with 0CPC diet showing normal hepatocytes with nucleus (Black arrow). The erythrocytes (E) are also seen.



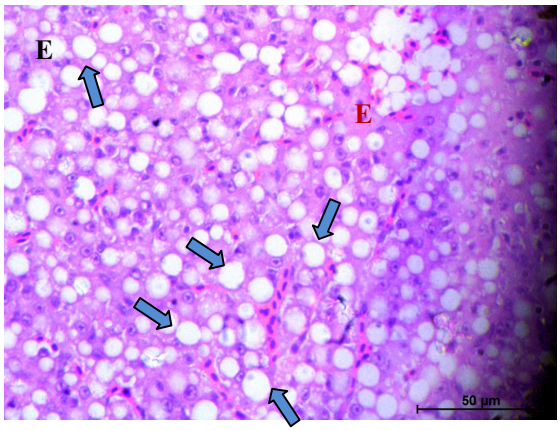
Liver section of snubnose pompano fed with diet containing 25CPC showing normal hepatocytes with nucleus.



Snubnose pompano fed with diet containing 50CPC showing normal hepatocytes and some hepatocytes with pyknotic nucleus (orange arrow) and the blood capillaries seen with mild congestion of erythrocytes. Very minimal hypertrophic nucleus (white arrow) also seen.



Liver section of fish fed with diet containing 75CPC showing mild vacculation of hepatocytes (Blue arrow) and pyknotic nucleus (orange arrow). Tissue lesions and damage of connective tissues also noticed.

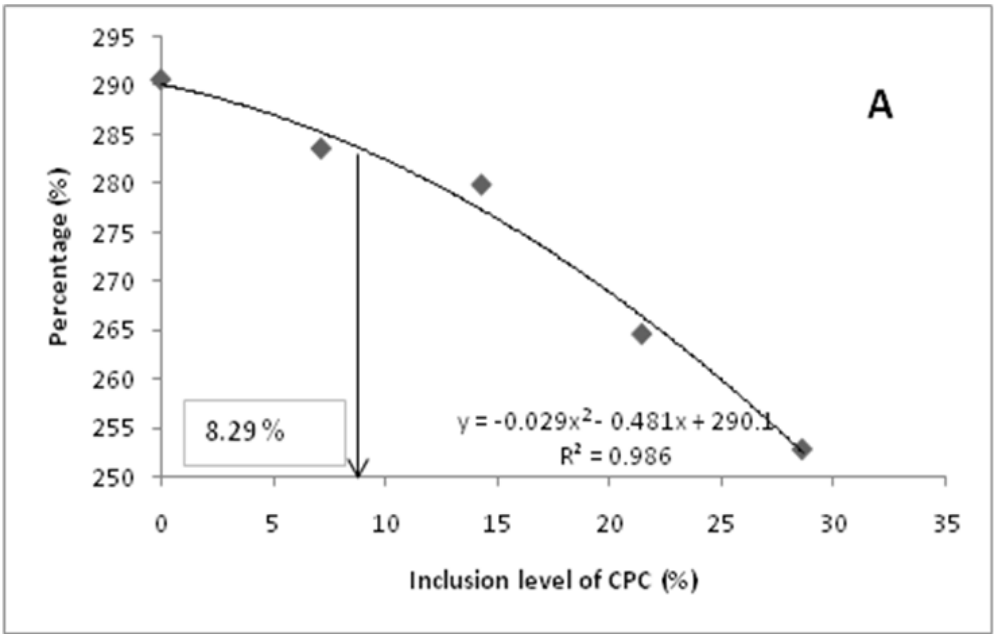


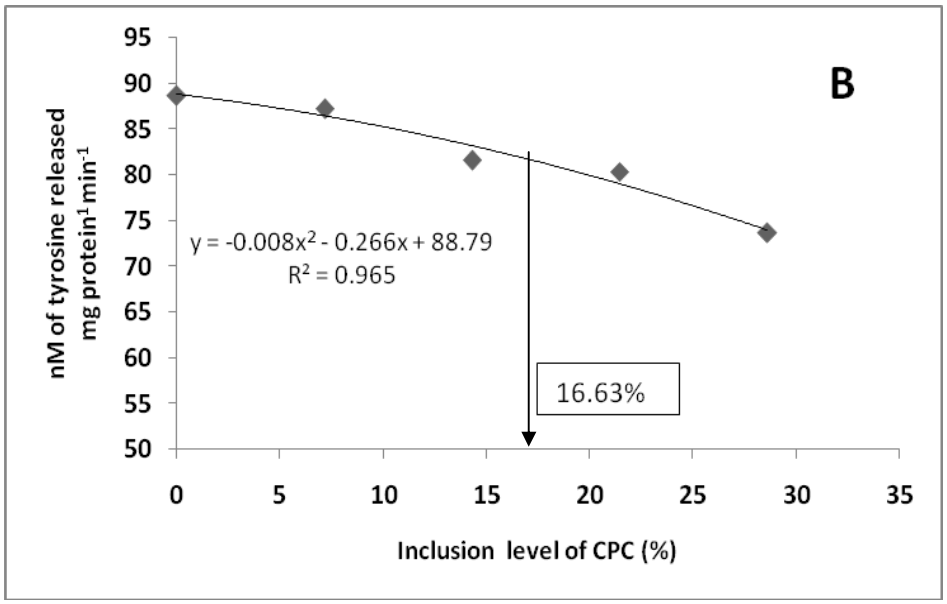
Liver section of 100CPC group fish showed mostly cytoplasmic round circumscribed vacuolation of hepatocytes (blue arrow) along with increased number of erythrocytes (E) were also seen.

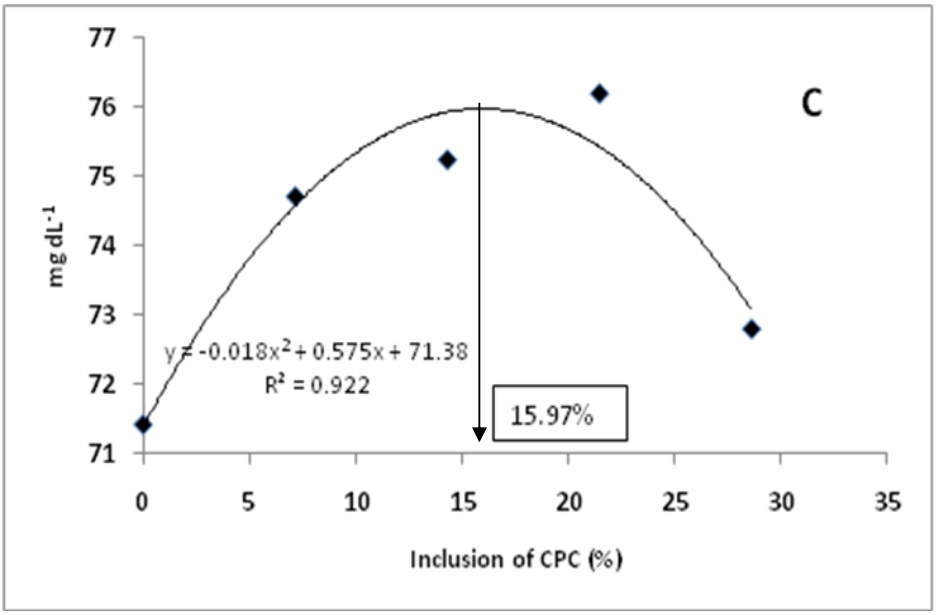
Fig. 17: The liver section of snubnose pompano fed with various levels of CPC to replace fishmeal in the diet. A) 0CPC; B. 25CPC; C. 50CPC; D. 75CPC and E. 100CPC treatments (stained with H&E; x40).

Dose optimization for inclusion of CPC in the diet

The second degree polynomial regression analysis of weight gain percentage, protease activity, serum glucose and serum total lipid level in snubnose pompano fed with varying levels of dietary CPC to replace fishmeal in the diet revealed that 8.29-17.28% is optimal for better growth, digestion and metabolism (Fig. 18).




B
nM of tyrosine released mg protein¹ min⁻¹
y = -0.008x² - 0.266x + 88.79
R² = 0.965
16.63%
Inclusion level of CPC (%)


C
mg dL⁻¹
y = -0.018x² + 0.575x + 71.38
R² = 0.922
15.97%
Inclusion of CPC (%)

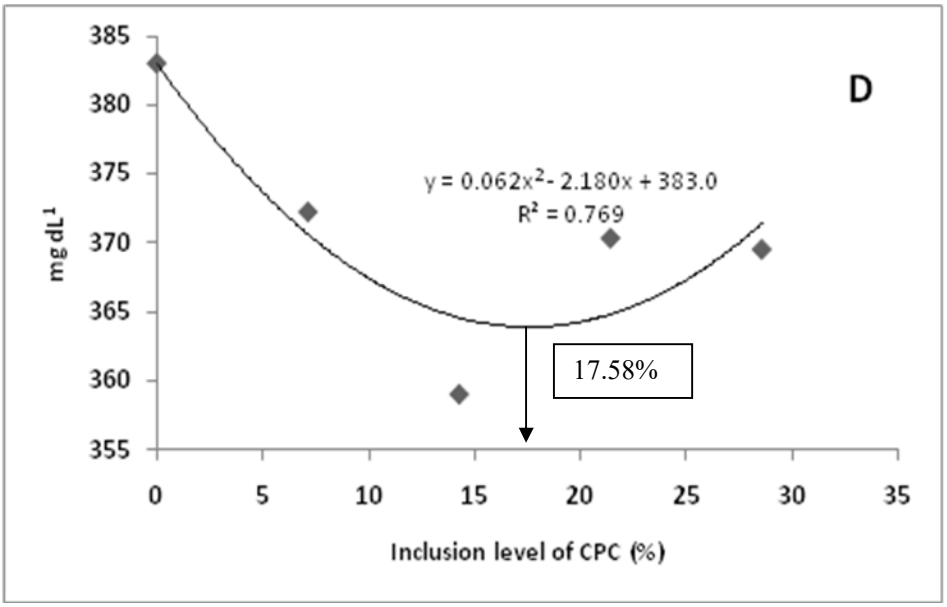


Fig. 18: Second degree polynomial regression analysis of weight gain percentage (A), protease activity (B), serum glucose (C) and serum total lipid level (D) in snubnose pompano fed with varying levels of dietary CPC to replace fishmeal in the diet.

The data expressed as arithmetic mean of three replicates.

Antioxidant defence system

The antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST) showed a significant difference ($P<0.001$) in liver (Table 18) and gill tissues (Table 19) among the treatments. As the inclusion of CPC increased in the diet, the SOD, CAT and GST levels were gradually increased, and a higher level was recorded in 100CPC group.

Table 18: The anti-oxidant enzyme activities in the liver tissue samples of experimental fishes fed with different experimental diets

Parameters	0CPC	25CPC	50CPC	75CPC	100CPC	df	F value	Level of significance
SOD	44.53 ^{a}±0.86	45.40 ^{a}±0.69	48.54 ^{b}±0.72	53.39 ^{c}±0.80	53.62^{c}±0.31	4	33.07	**
CAT	2.01 ^{a}±0.05	2.02 ^{a}±0.04	2.14 ^{b}±0.02	2.26 ^{b}±0.05	2.26 ^{b}±0.02	4	10.78	**
GST	0.27 ^{a}±0.008	0.28 ^{a}±0.006	0.32 ^{b}±0.005	0.33 ^{b}±0.003	0.35 ^{c}±0.003	4	39.73	**

Table 19: The anti-oxidant enzyme activities in the gill samples of experimental fishes fed with different experimental diets

Parameters	0CPC	25CPC	50CPC	75CPC	100CPC	df	F value	Level of significance
SOD	27.92 ^{a}±0.33	29.67 ^{ab}±0.62	31.48 ^{bc}±1.03	33.53 ^{cd}±0.88	34.46 ^{d}±0.80	4	12.11	**
CAT	1.04^{a}±0.01	1.08 ^{a}±0.04	1.17 ^{b}±0.02	1.19 ^{b}±0.02	1.26 ^{c}±0.01	4	15.41	**
GST	0.44^{a}±0.02	0.50 ^{b}±0.01	0.54 ^{c}±0.01	0.57 ^{c}±0.01	0.56 ^{c}±0.01	4	18.18	**

SOD, Superoxide dismutase; CAT, Catalase; GST, Glutathione-S-Transferase.

SOD specific activity is expressed in 50% inhibition of epinephrine auto oxidation mg protein^{-1} min^{-1}; GST specific activity is expressed as µmol mg protein^{-1} min^{-1}; Catalase specific activity is expressed in nanomoles H_2O_2 decomposed mg protein^{-1} min^{-1}.

Superscripted letters in the same row indicate intergroup statistical differences (Duncan's multiple range test) per level of replacement of FM using CPC: arithmetic means not sharing a common letter are significantly different (P<0·05) and arithmetic means without letters indicate a non significant difference (P>0.05).

The level of significance: ns indicates not significant, * indicates P<0.05 and ** indicates P<0.001.

Data expressed as arithmetic mean of three replicates ±SE

Study 3:

Effect of free gossypol on growth and immunity of snubnose pompano fed with varying level of dietary free gossypol

Growth estimation

The growth response in terms of weight gain percentage, SGR % and ADG does not show any significant difference (P>0.05) among the treatments (Table 20). However, higher weight grain percentage, SGR and ADG was observed in 0G group fed without gossypol in the diet. The feed utilization of snubnose pompano in terms of FCR, FE and PER showed significant difference among the treatments and higher FE was noticed in 0G group and higher PER was observed in 1G group. The lower and better FCR of 2.09 ±0.01 was observed in 0G treatment.

Hematological response:

The hematological responses of the experimental fishes fed with varying levels of free gossypol in the diet are given in the table 21. There was no significant difference (P>0.05) in the hematological response among the treatments. Higher levels of Hb, RBC, HCT and MCHC values were observed in 0G group. The WBC count was found higher in 1G treatment group followed by 0G treatment. The MCV and MCH values were higher in 2G treatment and the lowest value was witnessed in 0GR treatment.

Serum biochemical parameters

Serum total protein, albumin, globulin and A:G ratio showed significant difference among the treatments and higher values were observed in 0.2G group. The values showed a decreasing trend as the level of inclusion of free gossypol increased (Table 22).

Table 20: Growth response of experimental fishes fed with varying levels of free gossypol

Parameter	0G	0.2G	0.5G	1G	2G	Df	F value	Level of significance
Initial wt	11.75 ^{b}±0.05	11.58 ^{b}±0.05	11.15^{a}±0.07	11.33 ^{a} ±0.09	11.26 ^{a}±0.03	4	14.76	**
Final wt	24.78±0.20	24.34±0.48	23.33±0.86	23.35±1.49	22.69±0.10	4	3.50	ns
WG (%)	110.87±1.23	110.12±6.18	109.17±6.64	105.86±11.45	101.44±0.69	4	0.39	ns
SGR (%)	1.66±0.01	1.65±0.04	1.63±0.07	1.60±0.12	1.56±0.01	4	0.42	ns
ADG	0.29±0.01	0.28±0.01	0.27±0.02	0.27±0.03	0.25±0.01	4	0.70	ns
FCR	2.09 ^{a}±0.01	2.18 ^{bc}±0.01	2.13 ^{ab}±0.02	2.22 ^{c}±0.03	2.18 ^{bc}±0.04	4	4.75	*
FE	0.48 ^{c}±0.01	0.46 ^{ab}±0.01	0.47 ^{bc}±0.01	0.45 ^{a}±0.01	0.46 ^{ab}±0.01	4	4.88	*
PER	0.84 ^{a}±0.01	0.87 ^{bc}±0.01	0.85 ^{ab}±0.01	0.89 ^{c}±0.01	0.87 ^{bc}±0.02	4	4.71	*

WG (%), Weight gain percentage; SGR, Specific growth rate; ADG, Average daily growth; FCR, Feed conversion ratio; FE, Feed efficiency; PER, Protein efficiency ratio.

Superscripted letters in the same row indicate intergroup statistical differences (Duncan's multiple range test) per level of inclusion of gossypol: arithmetic means not sharing a common letter are significantly different (P<0·05) and arithmetic means without letters indicate a non significant difference (P>0.05).

The level of significance: ns indicates not significant, * indicates P<0.05 and ** indicates P<0.001.

Data expressed as arithmetic mean of three replicates ±SE

Table 21: Hematological responses of experimental fishes fed with varying levels of free gossypol

Parameter	0G	0.2G	0.5G	1G	2G	df	F value	Level of significance
Hb (g dL^{-1})	11.56±0.60	10.80±0.21	10.46±0.38	10.20±0.28	10.13±0.17	4	2.55	ns
WBC (10^3 cells mm^{-3})	5.50±0.17	4.76±0.26	4.60±0.43	5.63±0.31	4.60±0.32	4	2.60	ns
RBC (10^6 cells mm^{-3})	2.87±0.04	2.60±0.16	2.53±0.10	2.50±0.09	2.41±0.06	4	3.08	ns
HCT (%)	32.96±0.08	31.67±1.45	32.20±0.36	30.23±0.50	30.53±0.20	4	2.56	ns
MCV (fL)	114.93±1.99	122.59±9.82	127.24±3.74	121.12±2.59	127.07±3.7	4	0.94	ns
MCH (pg)	40.32±2.23	41.84±3.11	41.27±0.52	40.86±1.31	42.19±1.67	4	0.14	ns
MCHC (%)	35.09±1.90	34.19±1.03	32.48±0.84	33.74±0.96	33.18±0.37	4	0.75	ns

Hb, Hemoglobin; RBC, Red blood corpuscles; WBC, White blood corpuscles; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin; MCV, Mean corpuscular volume; PCV, Packed cell volume.

Superscripted letters in the same row indicate intergroup statistical differences (Duncan's multiple range test) per level of inclusion of gossypol: arithmetic means not sharing a common letter are significantly different (P<0·05) and arithmetic means without letters indicate a non significant difference (P>0.05).

The level of significance: ns indicates not significant, * indicates P<0.05 and ** indicates P<0.001.

Data expressed as arithmetic mean of three replicates ±SE.

Table 22: Serum protein profile of juveniles of snubnose pompano fed with varying levels of FG in the diet

Parameter	0G	0.2G	0.5G	1G	2G	df	F value	Level of significance
Total protein (g dL^{-1})	$4.54^{d} \pm 0.30$	$4.75^{cd} \pm 0.06$	$4.33^{bc} \pm 0.11$	$4.16^{b} \pm 0.09$	$3.78^{a} \pm 0.05$	4	23.63	**
Albumin (g dL^{-1})	$1.24^{c} \pm 0.03$	$1.17^{bc} \pm 0.02$	$1.14^{bc} \pm 0.04$	$1.19^{ab} \pm 0.01$	$1.07^{a} \pm 0.02$	4	5.66	*
Globulin (g dL^{-1})	$3.30^{c} \pm 0.03$	$3.58^{d} \pm 0.07$	$3.19^{c} \pm 0.08$	$2.97^{b} \pm 0.09$	$2.71^{a} \pm 0.04$	4	22.08	**
A:G ratio	$0.38^{bc} \pm 0.02$	$0.33^{a} \pm 0.01$	$0.36^{ab} \pm 0.01$	$0.40^{c} \pm 0.01$	$0.39^{c} \pm 0.01$	4	7.44	**

Superscripted letters in the same row indicate intergroup statistical differences (Duncan's multiple range test) per level of inclusion of gossypol: arithmetic means not sharing a common letter are significantly different (P<0·05) and arithmetic means without letters indicate a non significant difference (P>0.05).

The level of significance: ns indicates not significant, * indicates P<0.05 and ** indicates P<0.001.

Data expressed as arithmetic mean of three replicates ±SE

Serum lipid biochemical profile

The serum lipid biochemical profile is given in Fig. 19 and Fig. 20. Lipid biomolecules such as total lipid, triglycerides and VLDL showed significant difference among the treatments (P<0.001). However, serum cholesterol, phospholipids, HDL and LDL levels did not show significant difference among the treatments (P>0.05).

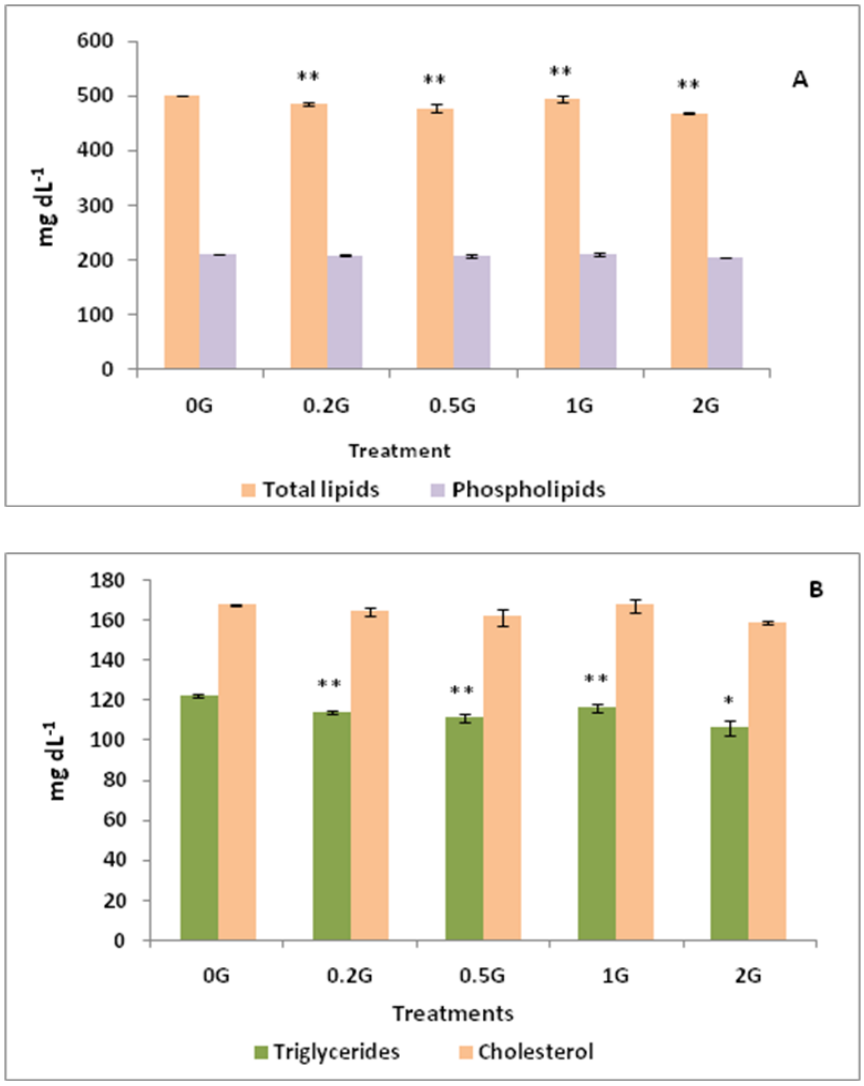


Fig . 19 : Serum total lipid and phospholipids (A), triglyceride and cholesterol (B), levels in experimental fishes fed with varying levels of dietary free gossypol

The level of significance: *significantly different from control at P<0.05 and ** indicates significantly different from control at P<0.001. Data expressed as arithmetic mean of three replicates ±SE. F value for total lipids and phospholipids are 6.63 and 2.08 respectively; F value for triglycerides and cholesterol are 7.90 and 2.08 respectively; df 4.

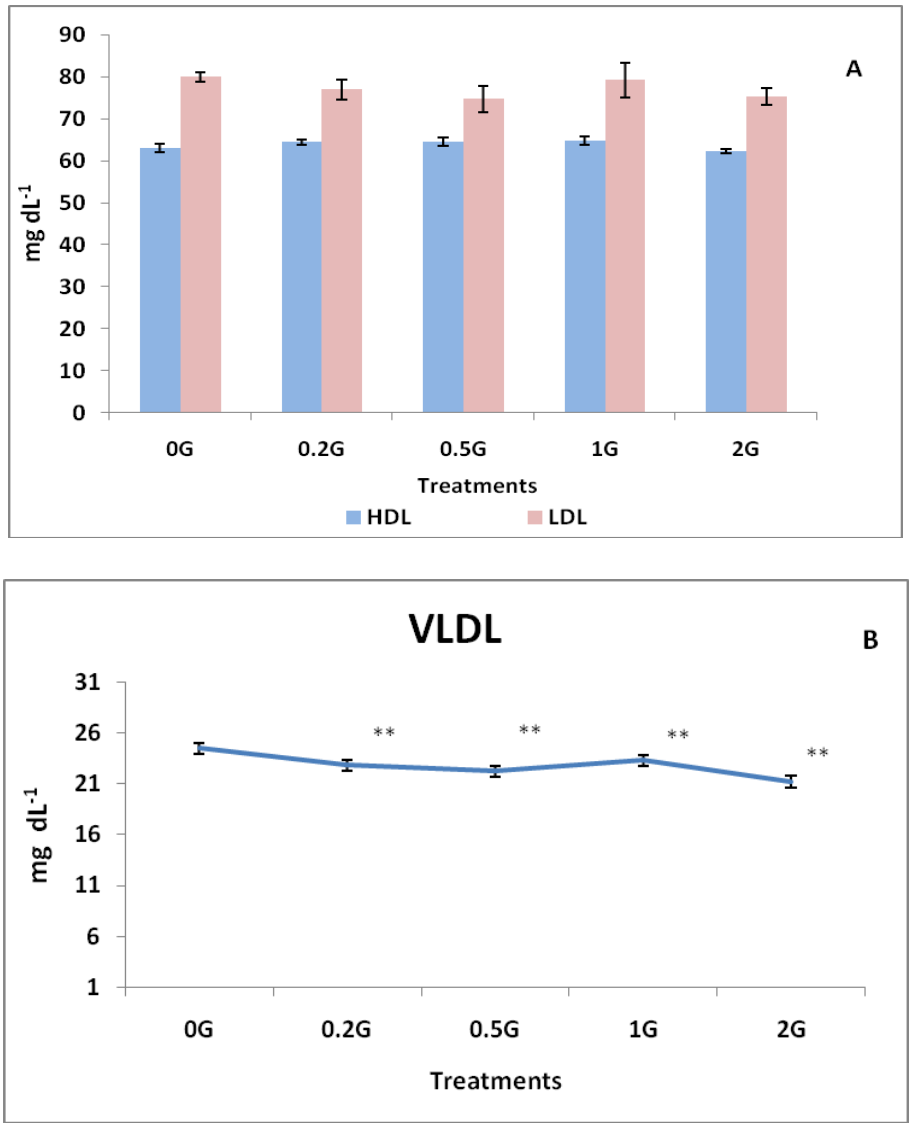


Fig. 20: Serum HDL & LDL (A) and VLDL (B) levels in experimental fishes fed with varying levels of dietary free gossypol

The level of significance: *significantly different from control at P<0.05 and ** indicates significantly different from control at P<0.001. Data expressed as arithmetic mean of three replicates ±SE. F value for HDL, LDL and VLDL are 1.63, 0.69 and 7.90 respectively; df 4.

Stress biomarkers:

AChE activity of brain samples and respiratory burst activity from different treatment groups of *T. blochii* juveniles showed significant difference (P<0.001) among the treatments (Fig. 21A). The respiratory burst activity showed significant difference (P<0.001) among the treatments and better RB activities were observed in 0G and 0.2G treatments (Fig. 21B).

Among the treatments, AChE and RB activities showed a decreasing trend as the level of gossypol inclusion increased in the diet.

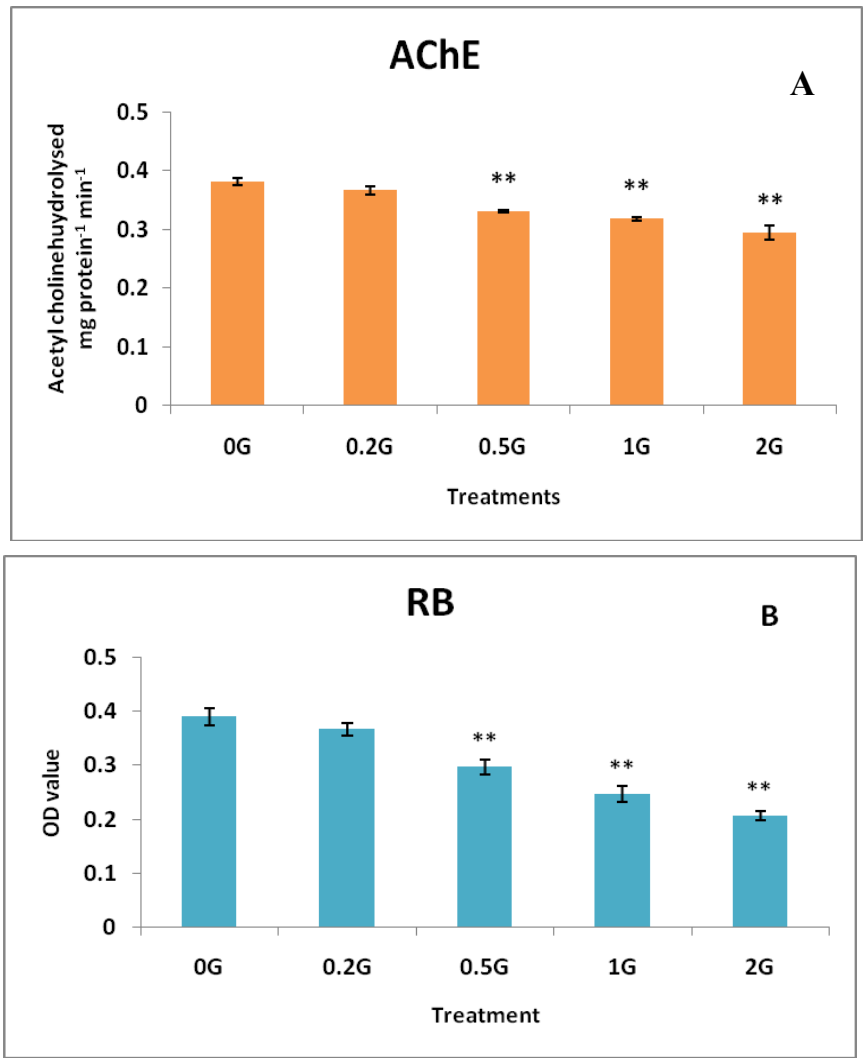


Fig 21: The stress biomarker levels in experimental fishes fed with varying levels of dietary free gossypol: AChE (A); respiratory burst activity (B)

The level of significance: *significantly different from control at P<0.05 and ** indicates significantly different from control at P<0.001. Data expressed as arithmetic mean of three replicates ±SE. F value for AChE and respiratory burst activity are 25.78 and 34.32 respectively; df 4.

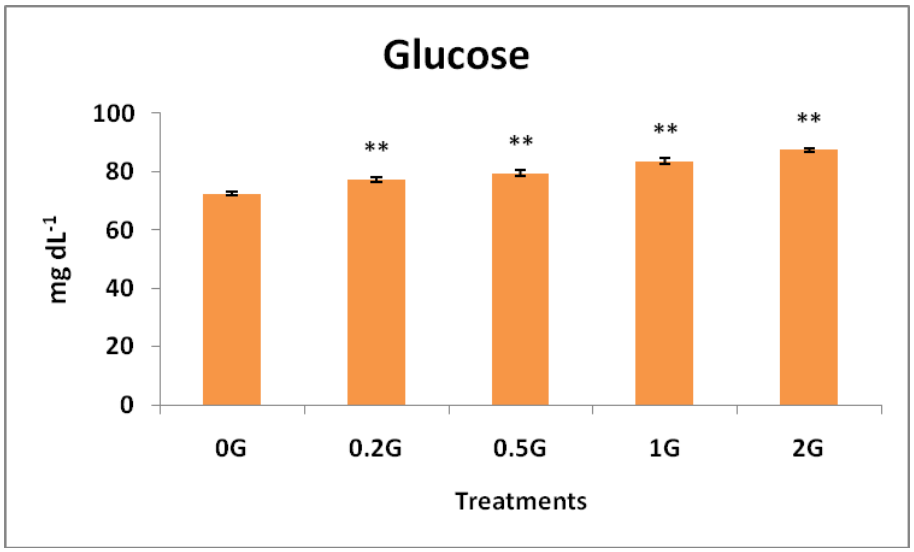


Fig 22: Serum glucose levels in experimental fishes fed with varying levels of dietary free gossypol

The level of significance: *significantly different from control at P<0.05 and ** indicates significantly different from control at P<0.001. Data expressed as arithmetic mean of three replicates ±SE. F value for serum glucose is 47.66; df 4.

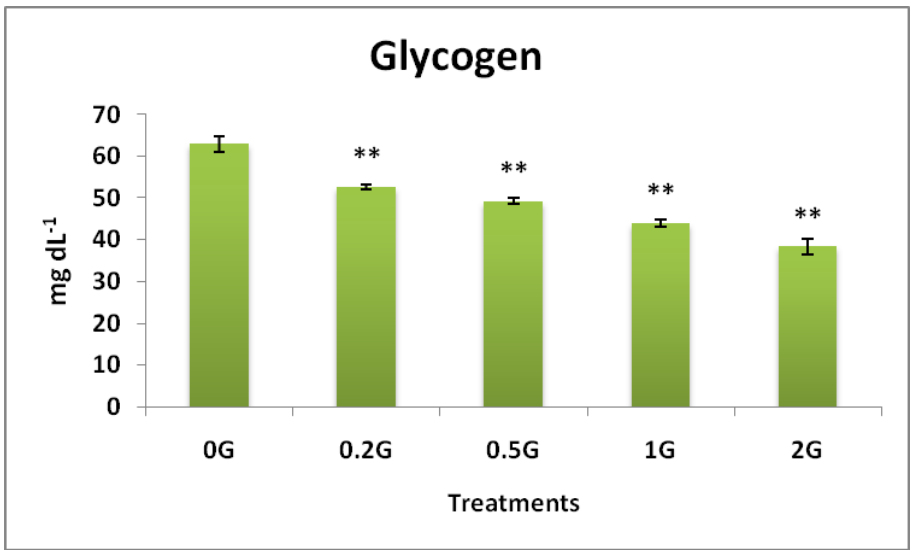


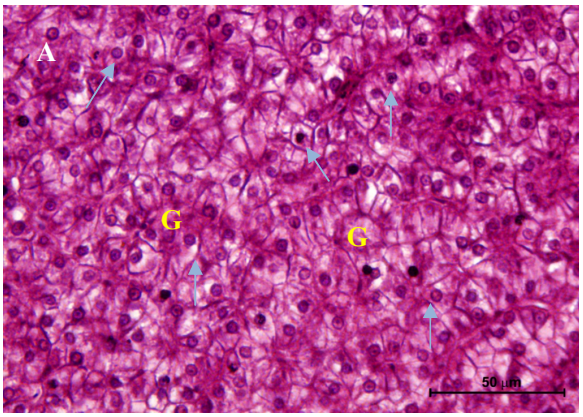
Fig 23: Liver glycogen levels in experimental fishes fed with varying levels of dietary free gossypol

The level of significance: *significantly different from control at P<0.05 and ** indicates significantly different from control at P<0.001. Data expressed as arithmetic mean of three replicates ±SE. F value for liver glycogen is 51.75; df 4.

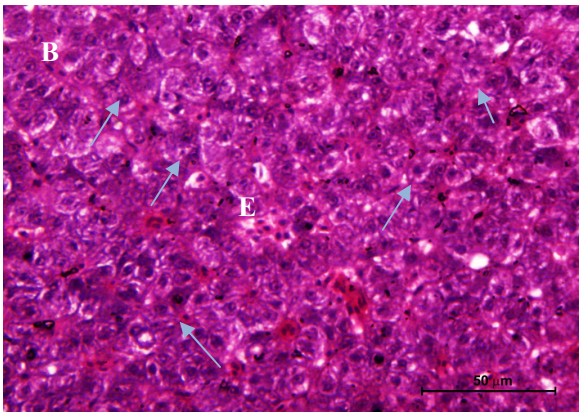
Serum glucose level showed an increasing trend (Fig. 22) and liver glycogen showed a decreasing trend as the inclusion of gossypol increased in the diet (Fig. 23). Both serum glucose and liver glycogen levels showed significant difference ($P<0.001$) among the treatments.

Liver histology

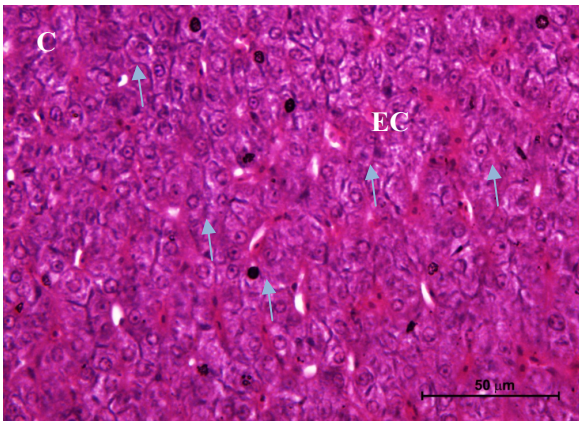
The detailed observation of morphological structure of liver sections of snubnose pompano fed with varying levels of free gossypol in the diet (Fig. 24).



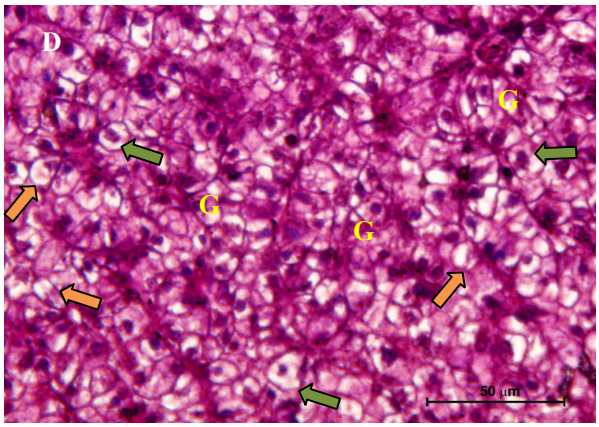
Liver section of snubnose pompano fed with control diet showing normal hepatocytes with nucleus. Glycogen deposition (G) was observed in some of the hepatocytes.



Liver section of snubnose pompano fed with diet containing 200 mg free gossypol kg^{-1} showing normal hepatocytes with nucleus. The erythrocytes (E) are normal.



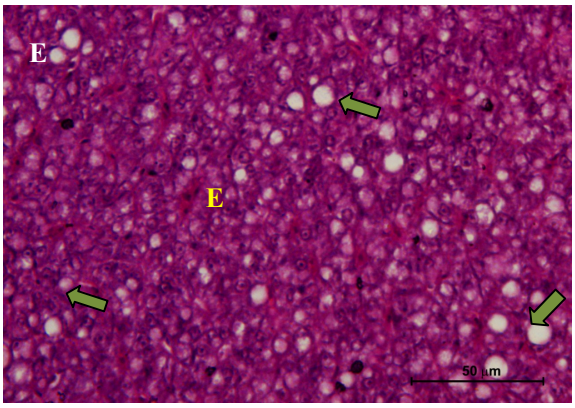
Snubnose pompano fed with diet containing 500 mg free gossypol kg^{-1} showing normal hepatocytes (blue arrow) and the blood capillaries seen with mild congestion of



Snubnose pompano fed with diet containing 1000 mg free gossypol kg^{-1} showing mild vacculation of hepatocytes (orange arrow) and pyknotic nucleus. Glycogen deposition (G) was

erythrocytes. Macrophages also seen.

observed in some of the hepatocytes and became hypertrophic (green arrow).



Liver section of 2G group fish showed cytoplasmic round circumscribed vacuolation of hepatocytes (green arrow). Few erythrocytes are also visible.

Fig. 24: The photomicrographs of liver sections of snubnose pompano fed with various levels of free gossypol in the diet. A) 0G; B). 0.2G; C). 0.5G; D). 1G and E). 2G treatments (stained with H&E; x40)

Phagocytic assay

There was significant difference (P<0.001) in phagocytic activity and phagocytic index among the experimental groups (Fig. 25).

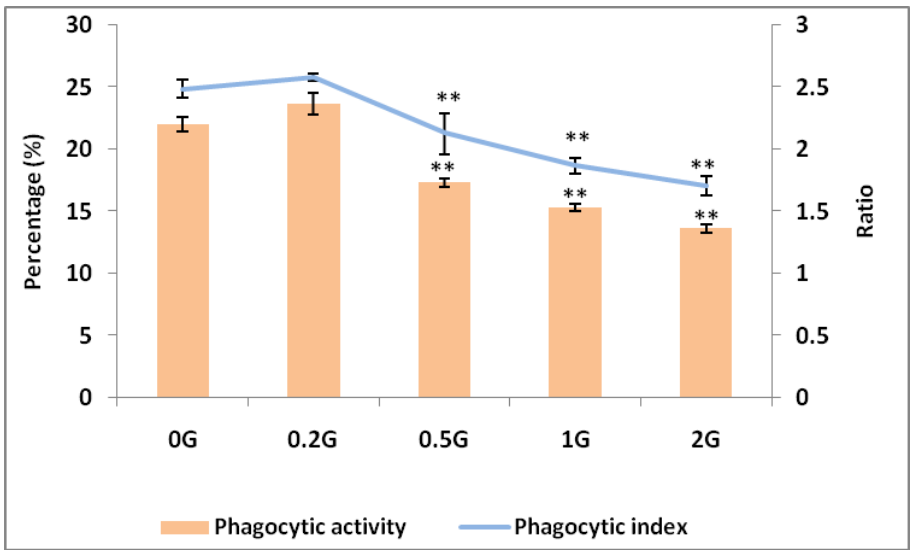


Fig 25: Phagocytic activity and phagocytic index in experimental fishes fed with varying levels of dietary free gossypol

The level of significance: *significantly different from control at P<0.05 and ** indicates significantly different from control at P<0.001. Data expressed as arithmetic mean of three replicates ±SE. F value for phagocytic activity and phagocytic index are 63.73 and 16.40 respectively; df 4.

The phagocytic activity and phagocytic index exhibited increasing trend until 200 mg FG in the diet and showed a decreasing trend as the level of FG content increased in the diet. The phagocytsing cells and bacterial cells are marked in the photomicrographs from different treatments (Fig. 26A-E).

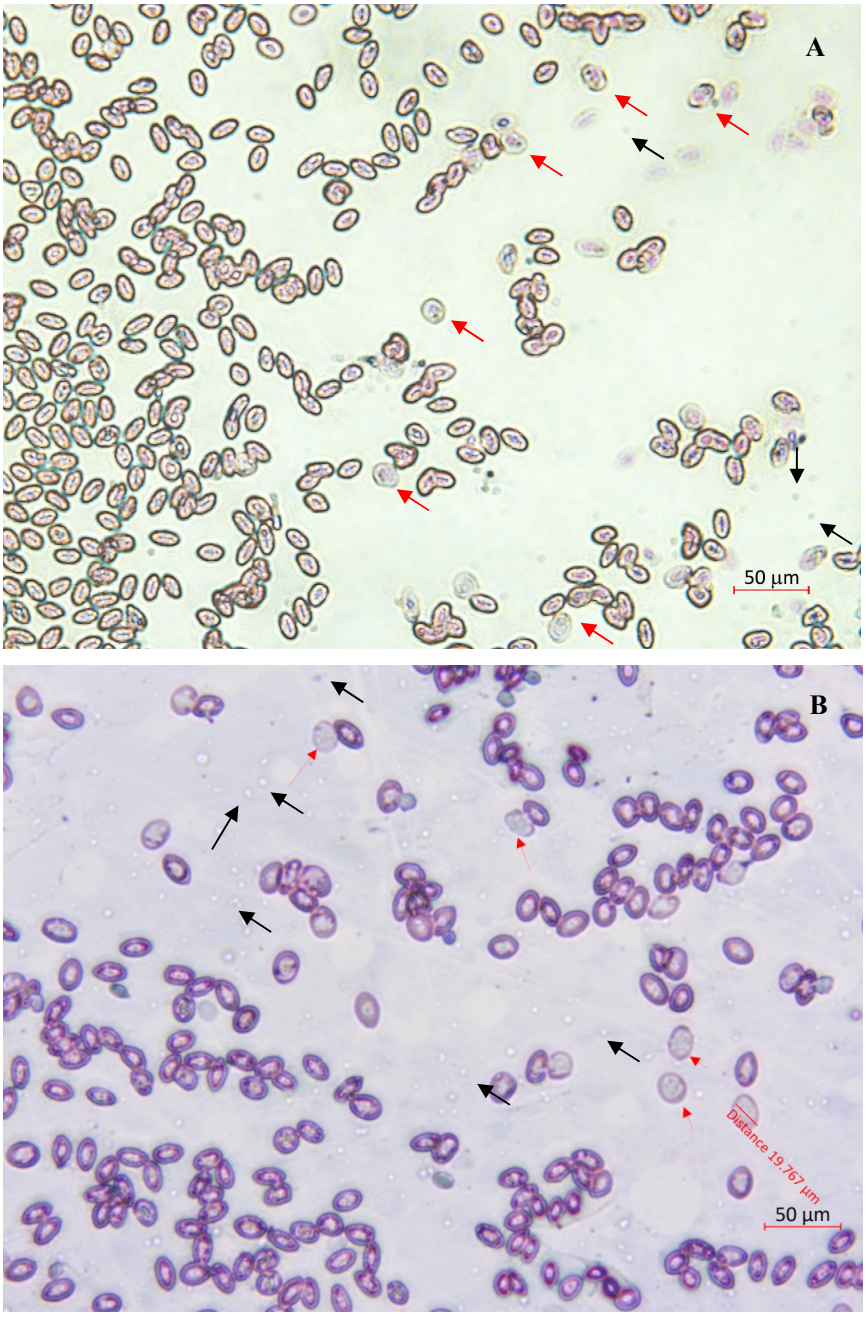
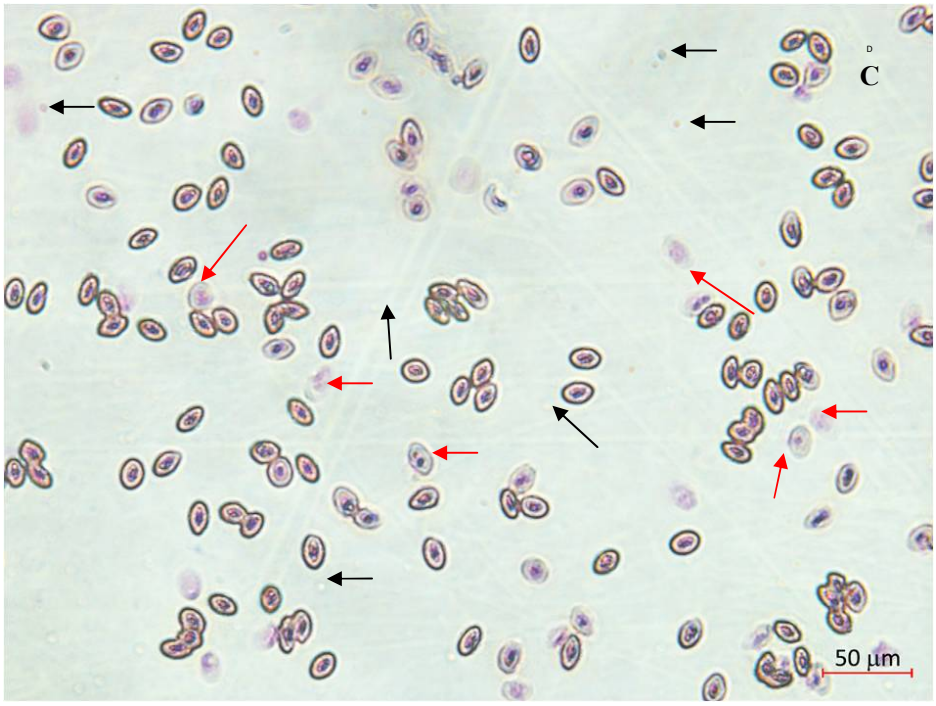


Fig. 26 (A, B): Photomicrographs of phagocytozing cells from different treatments (0G and 0.2G). The red colour arrow shows the neutrophils involving phagocytosis process. Black arrow indicates the bacterial cells. Stained with giemsa; x40.



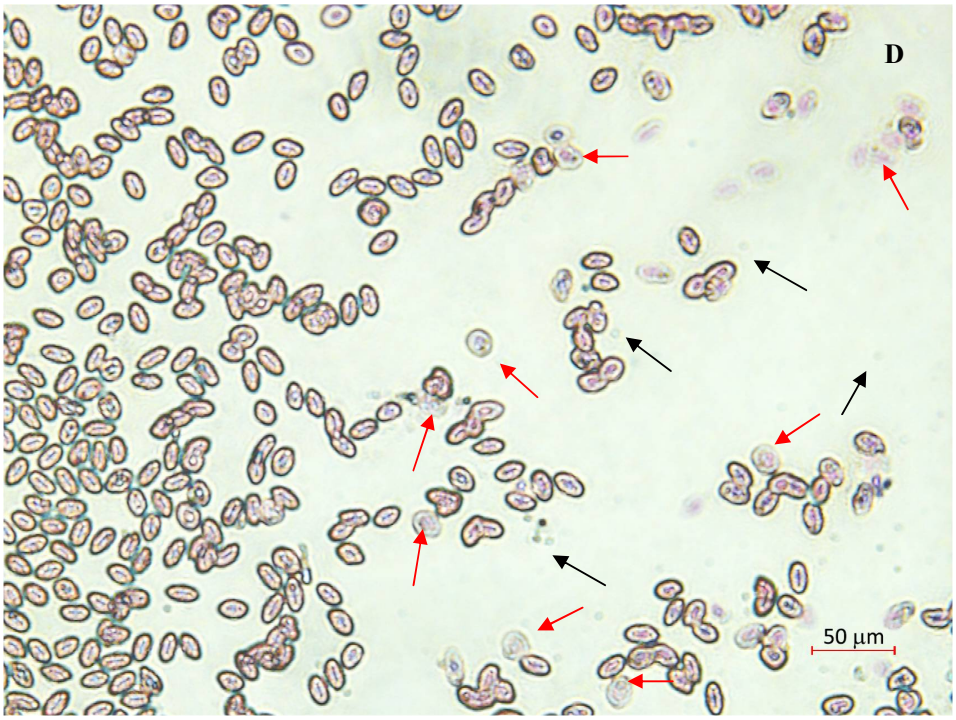


Fig. 26 C, D: Photomicrographs of phagocytozing cells from different treatments (0.5G& 1G)

The red colour arrow shows the neutrophils involving phagocytosis process. Black arrow indicates the bacterial cells. Stained with giemsa; x40.

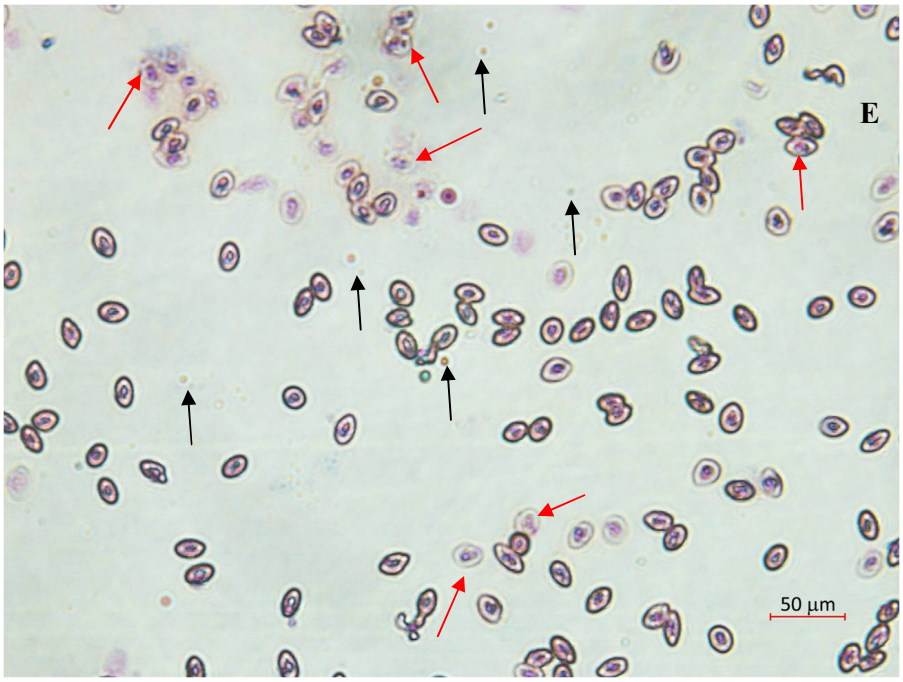


Fig. 26E: Photomicrographs of phagocytozing cells from 2G group

The red colour arrow shows the neutrophils involving phagocytosis process. Black arrow indicates the bacterial cells. Stained with giemsa; x40.

Relative percentage of survival

Among the different treatments better relative percentage of survival was observed in 0.2G group and followed by 0G group (Fig. 27). No significant difference in survival and mortality rate of snubnose pompano among the treatments (Table 23).

Table 23: Relative percentage of survival and survival rate in juveniles of snubnose pompano experimentally infected with *V. parahaemolyticus*

Treatment	Survival (%)	Mortality (%)	RPS (%)
0G	62.50±7.21	37.50±7.21	30.77
0.2G	66.67±4.16	33.33±4.16	38.46
0.5G	58.33±4.16	41.67±4.16	23.08
1G	54.14±4.16	45.86±4.16	15.38
2G	45.83±4.16	54.17±4.16	-
Df	4	4	-
F value	2.64	2.64	-
Level of significance	Ns	Ns	-

The level of significance: *significantly different from control at P<0.05; ** indicates significantly different from control at P<0.001 and ns indicates not significant. Data expressed as arithmetic mean of three replicates ±SE.

Kaplan–Meier survivorship curve

The Kaplan–Meier survivorship curve is the visual representation that shows the probability of an event (survival and mortality) for the population under investigation at a respective time interval. In the present study better survivorship was noticed in 0.2G group followed by 0G group.

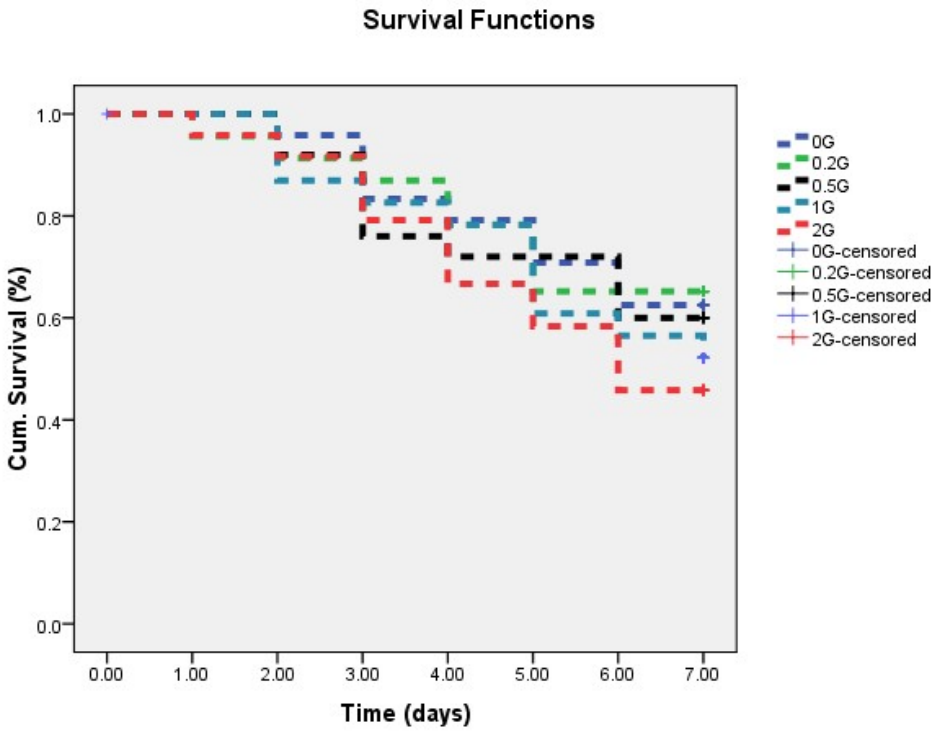


Fig. 27: Kaplan–Meier survivorship curves showing cumulative survival (%) over time (in days) for juveniles of snubnose pompano after experimentally infected with *V. parahaemolyticus*. The fish were fed with free gossypol incorporated diets (0, 0.2, 0.5, 1 or 2 g kg^{-1}) prior to bacterial challenge. Data for this challenge experiment is n = 24.

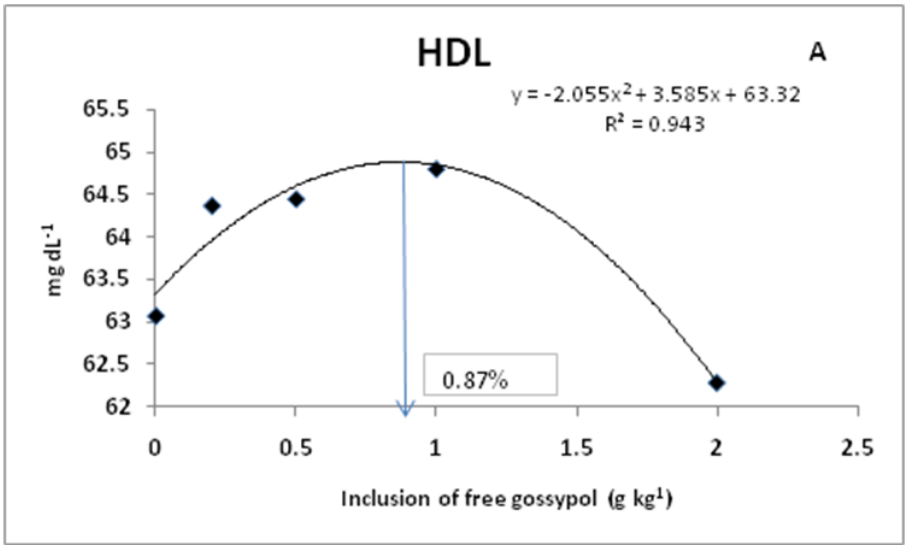
Immunomodulatory response of gossypol

At lower inclusion level of dietary gossypol (0.2 g kg^{-1}) exhibit better innate immunomodulatory response among the treatments which was evident from better phagocytic activity, phagocytic index, relative percentage of survival, serum total protein, globulin, acetylcholinesterase activity and respiratory burst activity. As the level of inclusion of dietary free gossypol increased the innate immune response is gradually declined in the juveniles of snubnose pompano.

Tolerance level of free gossypol in snubnose pompano

The second-degree polynomial regression analysis of serum lipid biochemical parameters such as total lipids and high-density lipoprotein levels (Fig. 28) indicates that

snubnose pompano can tolerate up to 1.2 g of free gossypol per kg diet without much adverse effect on general health.



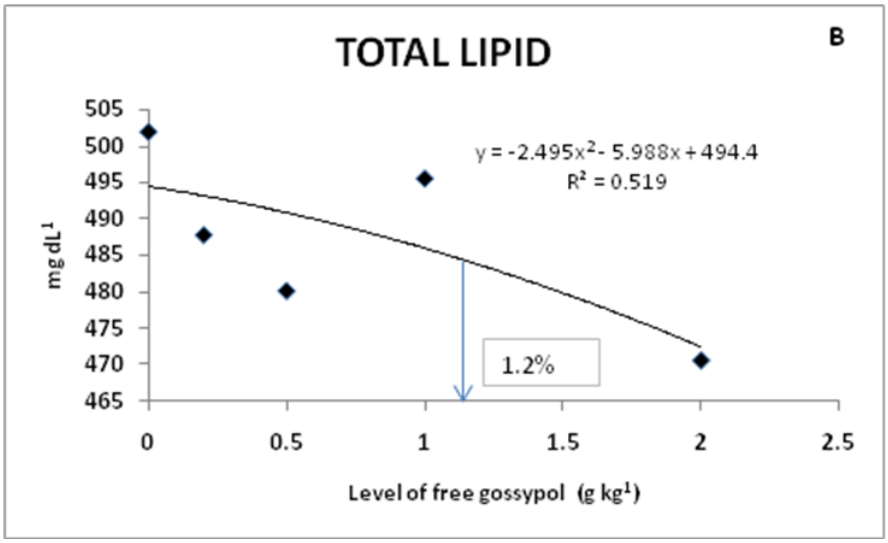


Fig 28: Second degree polynomial regression analysis of HDL (A) and total lipids (B) in snubnose pompano fed with varying levels of dietary free gossypol

The data expressed as arithmetic mean of three replicates ± SE.

7. DISCUSSION:

The growth performance of snubnose pompano in terms of WG%, SGR, ADG and TGC were found higher in control group followed by 100CSM containing 343 mg FG kg $^{-1}$ diet, which indicated that it can tolerate the FG than most of fishes and thus growth performance was not affected. This is in agreement with Cai et al. (2022) found that complete

replacement of fishmeal using plant protein ingredients (mainly cottonseed meal, soybean meal and rapeseed meal) in the diet of Gibel carp (*Carassius auratus gibelio*) supplementing with essential amino acids showed no significant different in growth and feed utilization.

According to Zheng et al. (2012) growth depression in fishes fed with CSM based diets are usually linked with the reduction in the bioavailable lysine contents of the diets. In the present study, the maximum inclusion level of CSM was 34.7% of diet which did not show any negative effects on growth due to supplementation of adequate level of lysine in the diet and availability of more quantity of arginine in the CSM replaced diets when compared with 0CSM diet. This is because the fact that FG present in CSM reacts with the free epsilon-amino groups from lysine and arginine to form bound gossypol which affects the digestibility of these amino acids (Broderick et al., 2013). In the same way, higher inclusion rate of 36.6%of CSM was reported for sub-adult stage of grass carp (Liu et al., 2016) without adverse effects on growth.

Aminotransferases mainly AST and ALT levels were found to vary based on the health status, environmental condition, stress, pollution and disease infection (Pan et al., 2003). Generally, stress and disease condition will elevate the AST and ALT levels and it is a very good indicator of liver health status. The reason behind this process is that fishes have a preference to use amino acids as major source of energy rather lipid and carbohydrates (Demeal, 1978) through enhanced gluconeogenesis and amino acid catabolism which occurs in liver (Perez-Jimenez et al., 2007). Lactate dehydrogenase is the terminal enzyme of the glycolysis pathway which converts pyruvate to lactate in muscle during anaerobic or less oxygen availability. Further, lactate is converted into pyruvate in liver during aerobic condition to continue the glycolytic cycle (Rogatzki et al., 2015). When the fish is under stress, anaerobic pathway is switched, and high levels of lactate is produced as it is the preferred substrate for gluconeogeneis to cope with stress for glucose production and thus LDH activity is increased (Chatterjee et al., 2006). Malate dehydrogenase is playing a key role in gluconeogenesis and lipogenesis that catalyzes the reversible oxidation of malate to oxalacetatein the cytosol (Panepucci et al., 2000) and an increased MDH activity in fishes indicates that incidence of greater amount of Kreb's cycle due to increased energy demand to cope with endured stress (Kumar et al., 2011b).

Alkaline phosphatase and acid phosphatase are membrane enzymes that plays an important role in the metabolism of phosphorus and thus involved in bone formation in higher vertebrates (Molina et al., 2005). Alkaline phosphatase is also helping to modify the

surface structure of the pathogen for improved recognition of pathogens for phagocytosis (Hu et al., 2008). Acid phosphatase from liver tissue is involved in the degradation of phagotised pathogens and indicates the ability of macrophages to digest the ingested bacteria and parasites and thus prevent their growth and multiplication in the host (Attwood et al., 1996).

In the current study, the activities of AST, ALT and ALP in the FM replaced using CSM groups were found slightly lower than the fishes fed with control diet without cottonseed meal (0CSM). This is agreement with Bu et al. (2017b) who reported similarly lower hepatic AST and ALT enzyme activities in juvenile Ussuri catfish fed with FM replaced using CSM in the diet. Wang et al. (2014) reported that the AST and ALP activities showing a fluctuating trend among the treatments and suggested that asynchronous effect of dietary CSM on their activities. The LDH and MDH activities were showing a decreasing trend as the level of inclusion of CSM increased in the diet. This result is in line with Rinchard et al. (2003) who reported similar trend of LDH activity as the level of CSM increased in the diet of male rainbow trout.

The levels of energetic metabolites such as triglycerides and cholesterol of fish are considered as important indices reflecting the nutritional and physiological status of fish (Xu et al., 2013) and body fat metabolism (Shen et al., 2020). Gossypol content of dietary CSM reduces the cholesterol in the body which may be attributed through reduced intestinal absorption of dietary cholesterol and may reduce the synthesis of LDL in the liver (Wang et al., 2009). In the present study, serum cholesterol, total lipid, LDL and VLDL contents of experimental fishes showing a declining trend as the level of inclusion of CSM increased in the diet which may be due to FG contents of the diets that reduced the intestinal absorption. This is in agreement with Liu et al. (2016) reported similarly lower level of cholesterol and LDL content in the pre-adults of grass carp fed with varying levels of CSM.

Dietary free gossypol (FG) accumulates higher amount in the liver and kidneys of animals (Wang et al., 2014) and in the muscle tissue the accumulation was very meagre that depends on the levels of dietary FG content and the nutritive profile of diet (Li and Robinson 2006). In the present study, the FG content of muscle tissues of snubnose pompano was at the maximum of 1.28 mg kg^{-1} body mass on as is basis in 100CSM which was insignificant while comparing the permissible limit of FG (450 mg kg^{-1}) in any cotton seed related products (FDA, 2021). This result is in accord with the results of Lee et al. (2006) who fed the rainbow trout with 58.8% dietary CSM for 3-years and concentration of FG in the muscle tissue was

lower than 1 mg kg^{-1} of wet tissue sample. Based on these results, it is evident that it is very safe to consume the fish fed with CSM based diets as the accretion was insignificant.

Histological changes of liver have been widely used as biomarkers for the evaluation of health status of fish exposed to contaminants, toxicants and pathogens (Thophon et al., 2003). The alterations in liver morphological structure and cytology significantly affect the normal liver function as liver is an excellent indicator of nutritional and physiological status of fishes (Xu et al., 2013). In the present study, the liver sections of experimental fishes did not show any serious gross structural changes even at higher FG content of 100CSM group except for the mild vacuolation of hepatocytes, few hypertrophic hepatocytes and few hepatocytes observed with pyknotic nucleus in 50CSM group. The present observation is in accord with the histological observations of Wang et al. (2014) who noticed that slight decrease in the volume of the hepatocytes with increased numbers in the juveniles of common carp.

The production of protein concentrate from cottonseed meal appears to be a viable option which will increase its protein content (at least 20-40 %), improve amino acid profile, protein digestibility and reduce the free gossypol content (nearly 70 %) and fibre content (Gerasimidis et al., 2007). In the present study, the CPC showed an increase of protein content (65%) and reduction of free gossypol content (79.73%). This is in agreement with Gerasimidis et al. (2007) and He et al. (2022) who found similar increase in protein content (26.5 and 80.5 % respectively) and reduction of gossypol content to 66% and 82.5% respectively.

The growth response in terms of WGP, SGR, ADG, TGC and DGC showing a decreasing trend beyond 25% of replacement of FM using CPC in the present study. This is in agreement with Shen et al. (2020) who found higher specific growth rate and weight gain (%) at 24% replacement of FM with CPC in the diet of *T. ovatus*. The reduction in growth of fish at higher inclusion level of plant proteins as a replacement of fishmeal may be because of the amino acid imbalance that can palpably hamper the growth performance of fish (Yao et al. 2020).

The activities of digestive enzymes are one of the critical indexes to estimate the digestive ability of digestive organs (Lee et al., 1984). Several researches indicate that dietary plant protein sources replacing the fish meal can affect the digestive responses in fishes (Liu et al., 2020; Shen et al., 2020). The protease, amylase and lipase activities of sunbnose pompano fingerlings showing a gradual declining trend as the level of inclusion of CPC

increased in the diet. This is in accord with the results of Liu et al. (2020) revealed that fishmeal replacement using dietary soybean meal showed decreased chymotrypsin, lipase and amylase activities as the level of inclusion increased.

Liver, kidney and gills of the fish are considered as the most important organs that controls vital activities such as respiration, metabolism, detoxification and excretion (Kurutaş et al., 2009). In liver, antioxidant activities are controlled by antioxidant enzymes produced by antioxidant gene expression (Javed et al., 2020). The SOD, CAT and GSH activities in the fishes fed with CPC by replacing FM showed lower values in 0CPC group and the values were increasing as the level of CPC inclusion increased may be due to presence of lower level of free gossypol that may excerpt innate immune response. This result is in line with Shen et al. (2020) who recorded similar response of SOD and GSH activity in the juveniles of *T. ovatus* fed with CPC. However, Xu et al. (2022) showed a fluctuating trend in largemouth bass fed with the diet containing varying levels of CPC. On the contrary, Liu et al. (2020) revealed that dietary soybean meal showed decreased CAT and SOD activities as the level of inclusion increased by substituting FM content which may be due to the presence of several anti-nutritional factors.

Intestine is the vital part of the digestive tract which plays a pivotal role in the digestion and absorption process of the fish (Jiajia et al., 2014). Intestinal damage may affect the digestion and absorption of nutrients that leads to abnormal growth of fish (Zhao et al., 2021). According to Geda et al. (2012), the height of the mucosal fold (MF) determined the intestinal absorptive area and the thickness of the lamina propria (LP) indicates the efficiency of the absorption process. Marine carnivorous fish larvae are very sensitive to the change in amino acid profile of diet with the replacement of FM using plant source proteins due to the fact that amino acids play crucial role in development of intestine and protein synthesis during the early stages (Geng et al., 2011; Jiang et al., 2016). In the present study, the thickness of LP has increased with a mild infiltration of neutrophils and size of goblet cells (GC) slightly increased in the fishes fed with higher inclusion of CPC (75CPC, 100CPC) in the diet. Similarly, Sitjà-Bobadillaa et al. (2005) reported that enterocytes of completely plant protein fed fishes showed an increased number of lipidic vacuoles and/or deposition of protein droplets, and the submucosa of intestine was dilated/hypertrophied and infiltrated with eosinophilic granular cells. Even though, the limiting amino acid levels were equally maintained in the different experimental diets used in this study, at complete replacement of FM using CPC, necrotic lesions noticed in the microvilli and detachment of surface epithelial

cells from underlying LP of fishes. This may be due to an imbalance of other amino acids that affects the intestinal morphology and malfunction of nutrient absorption process. This result is line with Chen et al. (2020) who reported significantly changed morphology in pearl gentian grouper as the inclusion level of CPC is increased beyond 24%. Many researchers illustrated that the structure of distal intestine of fish would be damaged when fed with high plant protein-contained diets in Asian seabass (Boonyaratpalin et al., 1998), rainbow trout (Merrifield et al., 2009), gilthead sea bream (Sitjà-Bobadillaa et al. 2005) and turbot (Peng et al., 2013).

The present study revealed that cottonseed protein concentrate can be used to replace 27.63-58.60 % of fishmeal without any adverse effect on growth, feed utilization and antioxidant response. Similarly, Shen et al. (2020) recorded the replacement of fishmeal with cottonseed protein concentrate in the diet of juvenile golden pompano was 25.93%.

Haematological status of fish is a useful indicator for monitoring fish health and physiological responses to any stressors (Ahmed et al., 2020). The FG may cause a reduction in Hct and Hb of fish (Mbahinzireki et al., 2001) and leukocyte counts which affect the immune-competence of the animal (Braga et al., 2012). The hemoglobin, RBC, HCT and MCHC levels were decreasing as the levels of FG increased in the diet. This is in agreement with Yildirim et al. (2003) who reported reduced hematocrit value, hemoglobin content and RBC content in channel catfish fed with higher amount of dietary FG of 900 mg/kg and or more (1200 and 1500 mg/kg). The decrease in the Hb and RBC count of fish fed with high dietary free gossypol may be due to the adverse reaction of FG with iron, and make it not available for erythropoiesis (Wang et al., 2014).

According to Xu et al. (2013), serum lipid metabolites namely triglycerides, cholesterol, HDL and LDL of fish are considered as essential biochemical indices that indicating the nutritional and physiological status of fish. Dietary free gossypol content has hypolipidemic effect and reduces the cholesterol, low density lipoprotesin (LDL) and very low density lipoprotein (VLDL) in the body through the reduced intestinal absorption of cholesterol and also limits the synthesis of LDL in the hepatic tissues (Shandilya and Clarkson, 1982). Higher serum total lipid, triglycerides, cholesterol, phospholipids, HDL, LDL and VLDL levels were witnessed in 0G group followed by 1G group and least values were noticed in 2G group. As the level of inclusion of dietary gossypol increases the lipid indices are reduced in this study. This is in accordance with results of Liu et al. (2016) who

reported similarly lower levels of cholesterol and LDL content in grass carp fed with varying levels of cottonseed meal with free gossypol content in the diet.

Serum protein profile in terms of higher albumin and globulin is a valuable indicator of better health status of fish through enhanced innate immune system function (Liang et al. 2018). The level of protein and globulin was decreased as the inclusion level of FG increased in the diet. This is in agreement with Sitjà-Bobadillaa et al. (2005) who found reducing level of plasma protein in gilthead sea bream fed with varying level of plant source proteins.

Acetylcholinesterase (AChE) is the key enzyme that responsible for catalysis of anti-inflammatory molecule acetylcholine (ACh) into choline and acetate during normal physiological condition (Das et al., 2009). During pathogenic bacterial infections, AChE plays the anti-inflammatory and immune modulatory function by down regulating its activity and thus the ACh level will increase to prevent the tissue damage due to inflammation (Da Silva et al., 2016). In the present study, AChE activity decreased as the level of inclusion of FG increased in the experimental diets. This is in agreement with Singh et al. (2006) reported lower levels of acetylcholine esterase in sheep fed Karinja oil Cake based diets than the deoiled ground nut concentrate fed animals.

According to Sharp and Secombes (1993), increased RB activity can be attributed to the increased bacterial pathogen killing activity of phagocytes as well as its count and accelerated macrophage phagocytosis. The destruction of ingested microorganisms by phagocytosis may be due to degranulation and metabolic activation when the generation of toxic intermediates of oxygen reduction occurs. In the present study, as the level of inclusion of FG increased the RB activity was decreased and higher RB activity was observed at 0.2G treatment which indicates its better pathogen killing ability and functioning as immunostimulant. This is in agreement with Yang et al. (2004) who found similar reduction of RB activity while replacing fishmeal using either meat and bone meal or poultry meal in the diet of *Macrobrachium nipponense.* Talpur et al. (2013) in Asian sea bass fed with dietary ginger showed higher level of RB activity and resistance against *V. harveyi.*

Glycogen content of vital organs are changeable as it is spent when required by the body and restored again while extra glucose intake through diet (Scarabello et al., 1991). Fish utilize glycogen as a main energy source during fasting and stress condition through the phosphorylation of glycogen phosphorylase that activates the glycogenolysis pathway to produce endogenous glucose by the liver (Enes et al., 2009).The liver glycogen content of control group was found higher than the FG fed groups which reveals that stress condition

increase the metabolism of glycogen through glycogenolysis to cope with increased energy demand. This result is comparable with reports of Kumar et al. (2015) in *Labeo rohia* fingerlings subjected to thermal stress showed lower liver glycogen content than the unstressed fishes.

According to Amend (1981), higher the relative percentage of survival indicates the positive effect of the administrated vaccine or compound. This supports the present result where the RPS of snubnose pompano was found higher in 0.2G that clearly elucidated that at lower concentration inclusion of FG through feed enhances the immunity and better protection against *V. parahaemolyticus* infection. This is corroborating the results of Gopalakannan and Arul (2010), in *C. carpio* showed enhanced innate immunity and better RPS (above 50%) against *A. hydrophila* infection through oral administration of β-glucan and whole cell yeast.

8. THE OUTCOME

This outcome of the research paves another path to reduce the fishmeal inclusion in the diet of snubnose pompano using cottonseed meal to formulate economically viable eco-friendly plant protein based aquafeed. This will reduce the pressure on fishmeal and offer value addition for a non-conventional feed resource (cottonseed meal).

The study also developed the methodology for the production of protein rich cottonseed protein concentrate which significantly reduces the free gossypol content in the diet. As the protein content of CPC is high that allows the inclusion of relatively less protein ingredients in marine carnivorous fish feed which otherwise will not be able to include in the high protein marine fish diet.

The results of the present study also offer insights on the level of tolerance of gossypol content in the diets of marine food fish, snubnose pompano and gives information on utility of free gossypol as an immunostimulant for enhancing innate immunity of fishes for sustainable mariculture.

9. CONCLUSION

The current study recommends the complete replacement of FM in the diet of snubnose pompano sans adverse effect on growth, nutritive profile, nutrient utilization, muscle quality and health of fish. The nutritive profile of cotton seed protein concentrate

developed showed enhanced protein and amino acid composition than the CSM and significantly reduced the anti-nutritional factor, i.e., free gossypol content. This makes cottonseed protein concentrate as a suitable high protein alternate ingredient for fishmeal replacement in the diet of marine fishes. The second degree polynomial regression analysis of valuable indicators in snubnose pompano fed with varying levels of dietary CPC to replace fishmeal in the diet revealed that 8.29-17.28% of CPC is optimal for better growth, digestion and metabolism. Based on the second-degree polynomial regression analysis of some serum lipid biochemical parameters indicates that snubnose pompano can tolerate up to 1.2 g of free gossypol per kg diet sans adverse effect on general health of the fish. It is also evident that at lower concentration (0.2 g kg^{-1}), free gossypol enhances the innate immune response in snubnose pompano. The long-term effect of free gossypol may be warranted for further utilization of CSM with high free gossypol content

10. REFERENCE

Agbo, N.W. (2008). Oilseed meals as dietary protein sources for juvenile Nile tilapia (*Oreochromis niloticus* L.). PhD Thesis. University of Stirling, Scotland, UK.

Ahmed, I., Reshi, Q.M. and Fazio, F. (2020). The influence of the endogenous and exogenous factors on hematological parameters in different fish species: a review. Aquaculture International 10, 1-31. https://doi.org/10.1007/s10499-019-00501-3

Amend, D.F. (1981). Potency testing of fish vaccines. Developments in Biological Standardization 49, 447–454.

Anderson, A.D., Alam, M.S., Watanabe, W.O., Carroll, P.M., Wedegaertner, T.C. and Dowd, M.K. (2016). Full replacement of menhaden fish meal protein by low-gossypol cottonseed flour protein in the diet of juvenile black sea bass *Centropristis striata*. Aquaculture 464, 618-628.

Anderson, D.P. and Siwicki, A.K. (1995). Basic haematology and serology for fish health programmes. In: Shhariff, M., Arthur, J.R. and Subasinghe, R.P. (Eds.), Diseases in Asian Aquaculture II. Fish Health section, Asian Fisheries society, Manila, Philippines, pp. 185-202.

AOAC (Association of Official Analytical Chemists), (2000). Ash of Animal Feed (942.05), Official Methods of Analysis of AOAC International, 17th edn. Association of Official Analytical Chemists International, Gaithersburg, MD, USA.

AOAC (Association of Official Analytical Chemists), (2000). Moisture of Animal Feed (934.01), Official Methods of Analysis of AOAC International, 17th edn. Association of Official Analytical Chemists International, Gaithersburg, MD, USA.

Attwood, E.M., Weich, D.J. and Oosthuizen, J.M. (1996). The influence of carbon particles on the concentration of acid phosphatase and lysozyme enzymes within alveolar macrophages during the killing and degradation of *Mycobacterium bovis*. Tuber Lung Disease 77, 341–347.

Barros, M.M., Lim, C. and Klesius, P.H. (2002). Effect of soybean meal replacement by cottonseed meal and iron supplementation on growth, immune response and resistance of channel catfish (*Ictalurus punctatus*) to *Edwarsiella ictaluri* challenge. Aquaculture 207, 263–279.

Bian, F., Jiang, H., Man, M., Mai, K., Zhou, H., Xu, W. and He, G. (2017). Dietary gossypol suppressed postprandial TOR signaling and elevated ER stress pathways in turbot

(*Scophthalmus maximus* L.). American Journal of Physiology- Endocrinolology and Metabolism 312, E37-E47.

Blaxhall, P.C. and Daisley, K.W. (1973). Routine haematological methods for use with fish blood. Journal of Fish Biology 5, 771-781.

Boonyaratpalin, M., Suraneiranat, P. and Tunpibal, T. (1998). Replacement of fish meal with various types of soybean products in diets for the Asian sea bass, *Lates calcarifer*. Aquaculture 161, 67-78.

Braga, A.P., Maciel, M.V., Guerra, D.G., Maia, I.S., Oloris, S.C. and Soto-Blanco, B. (2012). Extruded-expelled cottonseed meal decreases lymphocyte counts in male sheep. Revista de Medicina Veterinaria 163,147–152.

Broderick, G.A., Kerkman, T.M., Sullivan, H.M., Dowd, M.K. and Funk, P.A. (2013). Effect of replacing soybean meal protein with protein from upland cottonseed, Pima cottonseed, or extruded Pima cottonseed on production of lactating dairy cows. Journal of Dairy Science 96, 2374-2386. doi:10.3168/jds.2012-5723

Bu, X., Chen, A., Lian, X., Chen, F., Zhang, Y., Muhammad, I., Ge, X. and Yang, Y. (2017b). An evaluation of replacing fish meal with cottonseed meal in the diet of juvenile Ussuri catfish *Pseudobagrusus suriensis*: Growth, antioxidant capacity, nonspecific immunity and resistance to *Aeromonas hydrophila*. Aquaculture 479, 829-837.

Bu, X., Cheng, Y., Wang, C., Chen, J., Zhu, J. and Zhu, A. (2017a). Study on the gossypol removal from cottonseed meal by combination of biological and physical methods. Chinese Journal of Animal Science 53, 79-84.

Cai, C., Li, E., Ye, Y., Krogdahl, A., Jiang, G., Wang, Y. and Chen, L. (2011). Effect of dietary graded levels of cottonseed meal and gossypol on growth performance, body composition and health aspects of allogynogenetic silver crucian carp, *Carassius auratus gibelio*×*Cyprinus carpio*. Aquaculture Nutrition 17, 353-360.

Cai, W., Liu, H., Han, D., Zhu, X., Jin, J., Yang, Y. and Xie, S. (2022) Complete replacement of fishmeal with plant protein ingredients in Gibel carp (*Carassius auratus gibelio*) diets by supplementation with essential amino acids without negative impact on growth performance and muscle growth-related biomarkers. Frontiers in Marine Science https://doi.org/10.3389/fmars.2021.759086

Carver, R.H. and Nash, J.G. (2008). Doing data analysis with SPSS: version 16.0. Cengage Learning, Belmont, CA, USA.

Chakraborty, P., Mallik, A., Sarang, N. and Lingam, S.S. (2019). A review on alternative plant protein sources available for future sustainable aqua feed production. International Journal of Chemical Studies 7, 1399-1404.

Chatterjee, N., Pal, A.K., Das, T., Mohammed, M.S., Sarma, K., Venkateshwarlu, G. and Mukherjee, S.C. (2006). Secondary stress responses in Indian major carps *Labeo rohita* (Hamilton), *Catla catla* (Hamilton) and *Cirrhinus mrigala* (Hamilton) fry to increasing packing densities. Aquaculture Research 37, 472-476.

Chen, J., Liu, N., Zhang, H., Zhao, Y. and Cao, X. (2020). The effects of *Aeromonas hydrophila* infection on oxidative stress, nonspecific immunity, autophagy, and apoptosis in the common carp. Developmental & Comparative Immunology 105, 103587.

Cheng, H.N., He, Z., Ford, C., Wyckoff, W. and Wu, Q. (2020). A review of cottonseed protein chemistry and non-food applications. Sustainable Chemistry 1, 256–274.

Covaci, A., Voorspoels, S., Thomsen, C., van Bavel, B. and Neels, H. (2006). Evaluation of total lipids using enzymatic methods for the normalization of persistent organic pollutant levels in serum. Science of the Total Environment 366, 361-366.

Das, B.K., Debnath, C., Patnaik, P,, Swain, D.K., Kumar, K. and Misrhra, B.K. (2009). Effect of β-glucan on immunity and survival of early stage of *Anabas testudineus* (Bloch). Fish & Shellfish immunology 27, 678-683.

Da Silva, A.S., Boiago, M.M., Bottari, N.B., do Carmo, G.M., Alves, M.S., Boscato, C., Morsch, V.M., Schetinger, M.R.C., Casagrande, R.A. and Stefani, L.M. (2016). Hepatic cholinesterase of laying hens naturally infected by *Salmonella gallinarum* (fowl typhoid). Microbial Pathogenesis 98, 93-97.

Demeal, N.A. (1978). Some characteristics of carbohydrate metabolism in fish. Oceanis, DOC. Oceanography 4, 350-365.

Deng, J., Mai, K., Ai, Q., Zhang, W., Wang, X., Xu, W. and Liufu, Z. (2006). Effects of replacing fish meal with soy protein concentrate on feed intake and growth of juvenile Japanese flounder, *Paralichthys olivaceus*. Aquaculture 258, 503-513.

Doumas, B.T., Watson, W. and Biggs, H.G. (1971). Albumin standards and the measurement of serum albumin with bromcresol green. Clinica Chimica Acta 31, 87.

Drapeau, G. (1976). Protease from *Staphylococcus aureus*. Methods in Enzymology 45, 465–479.

Drew, M.D., Borgeson, T.L. and Thiessen, D.L. (2007). A review of processing of feed ingredients to enhance diet digestibility in finfish. Animal Feed Science and Technology 138, 118–136.

Duncan, D.B. (1955). Multiple Range and Multiple F-Test. Biometrics 11, 1-5.

Ellman, G.L., Courtney, K.D., Andres, V. and Feather-Stone, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology 7, 88-95.

Enes, P., Panserat, S., Kaushik, S. and Oliva-Teles, A.A. (2009). Nutritional regulation of hepatic glucose metabolism in fish. Fish Physiology and Biochemistry 35, 519-539.

FAO (2020). The State of World Fisheries and Aquaculture 2020. Rome, FAO.

Fawole, F.J., Sahu, N.P., Jain, K.K., Gupta, S. and Shamna, N. (2016). Protein isolate from rubber seed meal: preparation and evaluation. Journal of Experimental Zoology, India 19, 677-681.

FDA (2004). Bacteriological analytical manual online (http://www.cfsan.fda.gov/~ebam/ bam-toc.html), 8th edn, Revision A, 1998, Chapter 9. Arlington.

FDA (2021). Code of Federal Regulations Title 21, Modifed cottonseed products intended for human consumption sec tion 172.894, MD, USA. https://www.accessdata.fda.gov/ scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=172.894. Accessed 11 May 2021

Forster, L.A. and Cahloun, M.C. (1995). Nutrient values for cottonseed product deserve new look. Feed Stuffs 67, 1–5.

Fossati, P. and Prencipe, L. (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clinical Chemistry 28, 2077-2080.

Fournier, V., Huelvan, C. and Desbruyeres, E. (2004). Incorporation of a mixture of plant feedstuffs as substitute for fish meal in diets of juvenile turbot (*Psetta maxima*). Aquaculture 236, 451-465.

Friedewald, W.T., Levy, R.I. and Fredrickson, D.S., 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative centrifuge. Clinical Chemistry 18, 499–500.

Garen, A. and Levinthal, C. (1960). A fine structure genetic and chemical study of the enzyme alkaline phosphatase of *E. coli*. Biochimica et Biophysica Acta 38, 470-483.

Geda, F., Rekecki, A., Decostere, A., Bossier, P., Wuyts, B., Kalmar, I.D. and Janssens, G.P.J. (2012). Changes in intestinal morphology and amino acid catabolism in common carp at mildly elevated temperature as affected by dietary mannan oligosaccharides. Animal Feed Science and Technology, 178(1–2), 95–102.

Geng, M., Li, T., Kong, X., Song, X., Chu, W., Huang, R., Yin, Y. and Wu, G. (2011). Reduced expression of intestinal N-acetylglutamate synthase in suckling piglets: a novel molecular mechanism for arginine as a nutritionally essential amino acid for neonates. Amino Acids 40, 1513–1522.

Gerasimidis, K., Fillou, D.T., Babatzimcpoulou, M., Tassou, K. and Katsikas, H. (2007). Preparation of an edible cottonseed protein concentrate and evaluation of its functional properties. International Journal of Food Sciences and Nutrition 58, 486-490.

Glencross, B., Evans, D., Dods, K., McCafferty, P., Hawkins, W., Maas, R. and Sipsas, S. (2005). Evaluation of the digestible value of lupin and soybean protein concentrates and isolates when fed to rainbow trout, *Oncorhynchus mykiss*, using either stripping or settlement faecal collection methods. Aquaculture 245, 211-220.

Glibert, R. (2015). The role of a robust compound feed industry to meet the needs of a growing world population. World Aquaculture 2015, Jeju, Korea, 54 pp.

Gopakumar, G., Abdul Nazar, A.K., Jayakumar, R., Tamilmani, G., Kalidas, C., Sakthivel, M., Rameshkumar, P., Hanumnata Rao, G., Premjothi, R., Balamurugan, V., Ramkumar, B., Jayasingh, M. and Syde Rao, G. (2012). Broodstock development through regulation of photoperiod and controlled breeding of silver pompano, *Trachinotus blochii* (Lacepede, 1801) in India. Indian Journal of Fisheries 59, 53-57.

Gopalakannan, A. and Arul, V. (2010). Enhancement of the innate immune system and disease-resistant activity in *Cyprinus carpio* by oral administration of b-glucan and whole cell yeast. Aquaculture Research 41, 884-892.

Guo, M. (2009). Soy food products and their health benefits. In: *Functional foods: principles and technology* (Guo, M. (ed.), Woodhead Publishing., pp. 237-277. ISBN 9781845695927.

Habig, W.H., Pabst, M.J. and Jakoby, W.B. (1974). Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry, 249, 7130-7139.

Hardy, R.W. (2006). World fishmeal production outlook and the use of alternative protein meals for aquaculture. Avances en Nutrition Acuicola VII. (Ed. E.C. Suarez et al.,) VIII. Simposium International de Nutrition Acuicola, 15-17 Noviebre, Universidad Autonoma de Nuevo Leon, Mexico. pp. 410-419.

Hardy, R.W. (2010). Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. Aquaculture Research 41, 770-776.

Hardy, R.W. and Tacon, A.G.J. (2002). Fish meal: historical uses, production trends and future outlook for supplies. pp. 311–325. In: Stickney, R.R. and MacVey, J.P. (eds.) Responsible marine aquaculture. New York, CABI Publishing, 391 pp.

He, G., Zhang, T., Zhou, X., Liu, X., Sun, H., Chen, Y., Tan, B. and Lin, S. (2022). Effects of cottonseed protein concentrate on growth performance, hepatic function and intestinal health in juvenile largemouth bass, *Micropterus salmoides*. Aquaculture Reports 23, p.101052.

Hixson, S.M. (2014). Fish Nutrition and Current Issues in Aquaculture: The Balance in Providing Safe and Nutritious Seafood, in an Environmentally Sustainable Manner. Journal of Aquaculture Research and Development 5, 3.

Hron, R.J., Kim, H.L., Calhoun, M.C. and Fisher, G.S. (1999) Determination of (+)-,(−)-, and total gossypol in cottonseed by high-performance liquid chromatography. Journal of the American Oil Chemists Society 76, 1351-1355.

Hu, B., LI, X.Q., Leng, X.J., Li, J.L. and Wen, H. (2008). Effects of dietary vitamin C on growth, meat quality and non-specific immunity indices of grass carp, *Ctenopharyngodon idellus.* Journal of Fishery Sciences of China 15, 794–800.

Humason, G.L. (1979). Animal Tissue Techniques, 4th ed. W.H. Freeman and Company, San Francisco.

Javed, M., Abbas, K., Ahmed, T., Abdullah, S., Naz, H. and Amjad, H. (2020). Metal pollutants induced peroxidase activity in different body tissues of freshwater fish, *Labeo rohita.* Environmental Chemistry and Ecotoxicology 2, 162-167.

Jiajia, N., Qingyun, Y., Yuhe, Y. and Tanglin, Z. (2014). Factors influencing the grass carp gut microbiome and its effect on metabolism. FEMS Microbiology Ecology, 87, 704–714. https://doi. org/10.1111/1574-6941.12256

Jiang, J., Wu, X.Y., Zhou, X.Q., Feng, L., Liu, Y., Jiang, W.D., Wu, P. and Zhao, Y. (2016). Glutamate ameliorates copper-induced oxidative injury by regulating antioxidant defences in fish intestine. British Journal of Nutrition, 116, 70–79.

Kalidas, C., Sakthivel, M., Tamilmani, G., Ramesh Kumar, P., Nazar, A.K.A., Jayakumar, R.,Balamurugan, V., Ramkumar, B., Jothi, P.R. and Gopakumar, G. (2012). Survival and growth of juvenile silver pompano *Trachinotus blochii* (Lacepède, 1801) at different salinities in tropical conditions. Indian Journal of Fisheries 59, 95-98.

Karishma, R., Sahithya, U.L., Suneetha, P., Naik, V.C.B. and Krishna, M.S.R. (2016). Determination of total gossypol and free gossypol content in different varieties of Bt

and Non Bt Cotton seed extracts by High-Performance Liquid Chromatography (HPLC). Research Journal of Biotechnology 11, 70-74.

Katsivela, E., Kleppe, F., Lang, S. and Wagner, F. (1995). *Ustilago maydis* lipase I. Hydrolysis and ester-synthesis activities of crude enzyme preparation. Enzyme and Microbial Technology 17, 739–745.

Kaushik, S.J., Coves, D., Dutto, G. and Blanc, D. (2004). Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax.* Aquaculture 230, 391-404.

Kleemann, G.K., Dal Pai, M., Pezzato, L.E., Teixeira, C.P., Padovani, C.R. and Barros, M.M. (2011). Cottonseed meal as a substitute for soybean meal in Nile tilapia diet. Revista Brasileira de Saúde e Produção Animal 12, 805-818.

Kumar, K.S., Ganesan, K., Selvaraj, K. and Rao, P.V.S. (2014). Studies on the functional properties of protein concentrate of *Kappaphycus alvarezii* (Doty) Doty – an edible seaweed. Food Chemistry 153, 353–360.

Kumar, P., Pal, A.K., Sahu, N.P., Jha, A.K. and Priya, P. (2015). Biochemical and physiological stress responses to heat shock and their recovery in *Labeo rohita* fingerlings. The Proceedings of the National Academy of Sciences, India, Section B: Biological Sciences 85, 485-490.

Kumar, S., Sahu, N.P., Pal, A.K., Subramanian, S., Priyadarshi, H. and Kumar, V. (2011b). High dietary protein combats the stress of *Labeo rohita* fingerlings exposed to heat shock. Fish Physiology and Biochemistry 37, 1005–1019.

Kumar, V., Makkar, H.P.S. and Becker, K. (2011a). Evaluations of the nutritional value of *Jatropha curcas* protein isolate in common carp (*Cyprinus carpio* L.). Journal of Animal Physiology and Animal Nutrition 96, 1030-1043.

Kurutaş, E.B., Şahan, A. and Altun, T. (2009). Oxidative stress biomarkers in liver and gill tissues of spotted barb (*Capoeta barroisi* Lortet, 1894) living in Ceyhan River, Adana-Turkey. Turkish Journal of Biology 33, 275-282.

Lee, K.J., Rinchard, J., Dabrowski, K., Babiak, I., Ottobre, J.S. and Christensen, J.E. (2006). Long-term effects of dietary cottonseed meal on growth and reproductive performance of rainbow trout: three-year study. Animal Feed Science and Technology 126, 93-106.

Lee, P.G., Smith, L.L. and Lawrence, A.L. (1984). Digestive proteases of *Penaeus vannamei* Boone: Relationship between enzyme activity, size and diet. Aquaculture 42, 225–239.

Li, M.H. and Robinson, E.H. (2006). Use of cottonseed meal in aquatic animal diets: a review. North American Journal of Aquaculture 68, 14-22.

Liang, H., Mi, H., Ji, K., Ge, X., Re. M. and Xie, J. (2018). Effects of dietary calcium levels on growth performance, blood biochemistry and whole body composition in juvenile bighead carp (*Aristichthys nobilis*). Turkish Journal of Fisheries and Aquatic Science 18, 623-631.

Lim, S.J. and Lee, K.J. (2008). Supplemental iron and phosphorus increase dietary inclusion of cottonseed and soybean meal in olive flounder (*Paralichthys olivaceus*). Aquaculture Nutrition 14, 423-430.

Liu, H., Dong, X., Tan, B., Du, T., Zhang, S., Yang, Y., Chi, S., Yang, Q. and Liu, H. (2020). Effects of fish meal replacement by low-gossypol cottonseed meal on growth performance, digestive enzyme activity, intestine histology and inflammatory gene expression of silver sillago (*Sillago sihama* Forsskál) (1775). Aquaculture Nutrition 26, 1724-1735.

Liu, H., Yan, Q., Han, D., Jin, J., Zhu, X., Yang, Y. and Xie, S. (2016). Effect of dietary cottonseed meal on growth performance, physiological response, and gossypol accumulation in pre-adult grass carp, *Ctenopharyngodon idellus*. Chinease Journal of Oceanology and Limnology 34, 992-1003.

Lovatto, N.M., Loureiro, B.B., Pianesso, D., Adorian, T.J., Goulart, F.R., Speroni, C.S., Bender, A.B.B., Müller, J. and Silva, L.P. (2018). Sunflower protein concentrate and crambe protein concentrate in diets for silver catfish *Rhamdia quelen* (Quoy and Gaimard, 1824): use as sustainable ingredients. Anais da Academia Brasileira de Ciências 90, 3781-3790.

Luo, L., Xue, M., Wu, X., Cai, X., Cao, H. and Liang, Y. (2006). Partial or total replacement of fish meal by solvent-extracted cottonseed meal in diets for juvenile rainbow trout (*Oncorhynchus mykiss*). Aquaculture Nutrition 12, 418–424.

Lusas, E.W. and Rhee, K.C. 1995. Soybean protein processing and utilization, In: Practical handbook of soybean processing and utilization, (edited by D.R. Erickson), AOCS Press and the American Soybean Association, Champaign, 1995, pp. 117–160.

Makkar, H.P.S., Francis, G. and Becker, K. (2008). Protein concentrate from *Jatropha curcas* screw pressed seed cake and toxic and anti-nutritional factors in protein concentrate. Journal of the Science of Food and Agriculture 88, 1542–1548.

Marrufo-Estrada, D.M., Segura-Campos, M.R., Chel-Guerrero, L.A. and Betancur-Ancona, D.A. (2013). Defatted *Jatropha curcas* flour and protein isolate as materials for protein hydrolysates with biological activity. Food Chemistry 138, 77-83.

Martin, A. (1999). Aquaculture feed manufacturing practice in EU Mediterranean countries. Proaqua Nutrition S.A., Apto. Correos 16, Dueñas, E-34210, Palencia, Spain.

Martinez, W.H., Berardi, L.C. and Goldblatt, L.A. (1970). Cottonseed protein products. Composition and functionality. Journal of Agricultural and Food Chemistry 18, 961–968.

Mbahinzireki, G.B., Dabrowski, K., Lee, K.J., El-Saidy, D. and Wisner, E.R. (2001). Growth, feed utilization and body composition of tilapia (*Oreochromis* sp.) fed cotton seed meal-based diets in a recirculating system. Aquaculture Nutrition 7, 189-200.

Meiattini, F., Prencipe, L., Bardelli, F., Giannini, G. and Tarli, P. (1978). The 4-Hydroxybenzoate/4-aminophenazone chromogenic system used in the enzymic determination of serum cholesterol. Clinical Chemistry 24, 2161-2165.

Merrifield, D.L., Dimitroglou, A., Bradley, G., Baker, R.T.M. and Davies, S.J. (2009). Soybean meal alters autochthonous microbial populations, microvilli morphology and compromises intestinal enterocyte integrity of rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of fish diseases 32,755-766.

Misra, H.P. and Fridovich, I. (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry 247, 3170–3175 .

Molina, R., Moreno, I., Pichardo, S., Jos, A., Moyano, R., Monterde, J.G. and Cameán, A. (2005). Acid and alkaline phosphatase activities and pathological changes induced in Tilapia fish (*Oreochromis* sp.) exposed subchronically to microcystins from toxic cyanobacterial blooms under laboratory conditions. Toxicon 46, 725-735.

Nelson, N. and Somogyi, M. (1965) Determination of glucose. In: Oser BL (ed) Hawk's Physiological Chemistry, 14th edn. McGraw Hill Publication, New York, pp 1054–1055.

Ochoa, S. (1955). Malic dehydrogenase and malic enzyme, In: Caloric, S.P., Kaplan, N. (Eds.), Methods of Enzymology, Vol. I. Academic Press, New York, pp. 735-745.

Pan, C.H., Chien, Y,H. and Hunter, B. (2003). The resistance to ammonia stress of *Penaeus monodon* Fabricius juvenile fed diets supplemented with astaxanthin. Journal of Experimental Marine Biology and Ecology 297, 107-118.

Panepucci, L., Fernandes, M.N., Sanches, J.R. and Rantin, F.T. (2000). Changes in lactate dehydrogenase and malate dehydrogenase activities during hypoxia and after temperature acclimation in the armored fish, *Rhinelepis strigosa* (Siluriformes, Loricariidae). Revista Brasileira de Biologia 60, 353-360.

Peng, M., Xu, W., Ai, Q., Mai, K., Liufu, Z. and Zhang, K. (2013). Effects of nucleotide supplementation on growth, immune responses and intestinal morphology in juvenile turbot fed diets with graded levels of soybean meal (*Scophthalmus maximus* L.). Aquaculture 392, 51-58.

Pérez-Jiménez, A., Guedes, M.J., Morales, A.E. and Oliva-Teles, A. (2007). Metabolic responses to short starvation and refeeding in *Dicentrarchus labrax*. Effect of dietary composition. Aquaculture 265, 325-335.

Pike, I.H. and Barlow, S.M. (2003). Impact of fish farming on fish stocks. pp. 24–29. In: International aquafeed –directory and buyers' guide 2003. Luton, UK, Turret West Ltd, Bartham Press.

Prabu, D.L., Ebeneezar, S., Chandrasekar, S., Sayooj, P. and Vijayagopal, P. (2017). Indigenous Re-circulatory Aquaculture System (i-RAS) developed for fish nutrition research. Marine Fisheries Information Service: Technical & Extension Series 234, 20-21.

Rana, K.J., Siriwardena, S. and Hasan, M.R. (2009). Impact of rising feed ingredient prices on aquafeeds and aquaculture production (No. 541). Food and Agriculture Organization of the United Nations (FAO).

Reinhold, J.G. (1953). Manual determination of serum total protein, albumin and globulin fractions by Biuret method. In: Reiner, M. (Ed.), Standard Method of Clinical Chemistry. Academic Press, New York, pp. 88.

Rick, W. and Stegbauer, H.P. (1974). Amylase measurement of reducing groups. In: Bergmeyer, H.V. (Ed.), Methods of Enzymatic Analysis, Vol. 2, 2ndedn. Academic Press, New York, USA, pp. 885– 889.

Rinchard, J., Lee, K.J., Czesny, S. and Ciereszko, A. (2003). Effect of feeding cottonseed meal-containing diets to broodstock rainbow trout and their impact on the growth of their progenies. Aquaculture 227, 77–88.

Rinchard, J., Mbahinzireki, G., Dabrowski, K., Lee, K.J., Garcia-Abiado, M-A. and Ottobre, J. (2002). Effects of dietary cottonseed meal protein level on growth, gonad development and plasma sex steroid hormones of tropical fish tilapia *Oreochromis* sp. Aquaculture International 10, 11–28.

Rogatzki, M.J., Ferguson, B.S., Goodwin, M.L. and Gladden, L.B. (2015). Lactate is always the end product of glycolysis. Frontiers in Neuroscience 9, 22.

Scarabello, M., Wood, C.M. and Heigenhauseg, R.J.F. (1991). Glycogen depletion in juvenile rainbow trout as an experimental test of the oxygen debt hypothesis. Canadian Journal of Zoology 69, 2562–2568.

Seifter, S., Dayton, S., Novic, B. and Muntwyler, E. (1950). The estimation of glycogen with the anthrone reagent. Archives of Biochemistry and Biophysics 25, 191–200.

Shamna, N., Sardar, P., Sahu, N.P., Pal, A.K., Jain, K.K. and Phulia. V. (2015). Nutritional evaluation of fermented Jatropha protein concentrate in *Labeo rohita* fingerlings. Aquaculture Nutrition 21, 33–42.

Shandilya, L.N. and Clarkson, T.B. (1982). Hypolipidemic effects of gossypol in cynomolgus monkeys (*Macaca fascicularis*). Lipids 17, 285-290.

Sharp, G.J.E. and Secombes, C.J. (1993). The role of reactive oxygen species in the killing of the bacterial fish pathogen *Aeromonas salmonicida* by rainbow trout macrophages. Fish and Shellfish Immunology 3, 119-129.

Shen, J., Liu, H., Tan, B., Dong, X., Yang, Q., Chi, S. and Zhang, S. (2020). Effects of replacement of fishmeal with cottonseed protein concentrate on the growth, intestinal microflora, haematological and antioxidant indices of juvenile golden pompano (*Trachinotus ovatus*). Aquaculture Nutrition 26, 1119-1130.

Shepherd, J. (2012). Aquaculture: Are the Criticisms Justified? Feeding Fish to Fish. World Agriculture 3, 11–18.

Singh, P., Sastry, V.R.B., Garg, A.K., Sharma, A.K. and Agrawal, D.K. (2006). Effect of long-term feeding of expeller-pressed and solvent-extracted karanj (*Pongamia pinnata*) seed cake on the performance of lambs. Animal Feed Science and Technology, 126: 157–168.

Sitjà-Bobadilla, A., Peña-Llopis, S., Gómez-Requeni, P., Medale, F., Kaushik, S. and Pérez-Sánchez, J. (2005). Effect of fish meal replacement by plant protein sources on non-specific defence mechanisms and oxidative stress in gilthead sea bream (*Sparus aurata*). Aquaculture, 249, 387-400.

Smith, A.J., 1997. Post-column amino acid analysis, in: Smith, B.J. (Ed.), Methods in Molecular Biology, Vol. 64, Protein Sequencing Protocols. Humana Press, Totowa, NJ, pp. 139-146.

Stasiack, A.S. and Bauman, C.P. (1996). Neutrophil activity as a potent indicator of concomitant analysis. Fish and Shellfish Immunology 37, 539-546.

Sun, H., Tang, J., Yao, X., Wu, Y., Wang, X. and Liu, Y. (2016). Effects of replacement of fish meal with fermented cottonseed meal on growth performance, body composition and haemolymph indexes of Pacific white shrimp, *Litopenaeus vannamei* Boone, 1931. Aquaculture Research 47, 2623–2632.

Sun, H., Tang, J.W., Yao, X.H., Wu, Y.F., Wang, X., Liu, Y. and Lou, B. (2015). Partial substitution of fish meal with fermented cottonseed meal in juvenile black sea bream (*Acanthopagrus schlegelii*) diets. Aquaculture 446, 30-36.

Tacon, A.G.J. (2020). Trends in Global Aquaculture and Aquafeed Production: 2000–2017. Reviews in Fisheries Science & Aquaculture 28, 43-56.

Takahara, S., Hamilton, B.H., Nell, J.V., Kobra, T.Y., Oguna, Y. and Nishimura, E.T. (1960). Hypocatalesemia a new genesis carrier state. Journal of Clinical Investigation 29, 610–619.

Talpur, A.D., Ikhwanuddin, M. and Bolong, A-M.A. (2013). Nutritional effects of ginger (*Zingiberofficinale* Roscoe) on immune response of Asian sea bass, *Lates calcarifer* (Bloch) and disease resistance against *Vibrio harveyi.* Aquaculture 400-401, 46-52.

Thophon, S., Kruatrachue, M., Upathan, E.S., Pokethitiyook, P., Sahaphong, S. and Jarikhuan, S. (2003). Histopathological alterations of white seabass, *Lates calcarifer* in acute and subchronic cadmium exposure. Environmental Pollution, 121, 307-320.

Van Huis, A., Van Itterbeeck, J., Klunder, H., Mertens, E., Hallo-ran, A., Muir, G. and Vantomme, P. (2013). Edible insects – future prospects for food and feed security. Food and Agriculture Organization of the United Nations Forestry Paper 171. ISBN 978- 92-5-107595-1.

Wang, H., Johnson, L.A. and Wang, T. (2004). Preparation of soy protein concentrate and isolate from extruded-expelled soybean meals. Journal of American Oil Chemists' Society 81, 713-717.

Wang, X., Howell, C.P., Chen, F., Yin, J. and Jiang, Y. (2009) Gossypol-A polyphenolic compound from cotton plant. Advances in Food and Nutrition Research 58, 215-263.

Wang, X.F., Li, X.Q., Leng, X.J., Shan, L.L., Zhao, J.X. and Wang, Y.T. (2014). Effects of dietary cottonseed meal level on the growth, hematological indices, liver and gonad histology of juvenile common carp (*Cyprinus carpio*). Aquaculture 428, 79–87.

Wootton, I.D.P. (1964). Microanalysis in Medical Biochemistry, 4th edn. Grune & Stratton, New York, USA.

Wroblewski, F. and La Due, J.S. (1955). Lactic dehydrogenase activity in blood. Proceedings of the Society for Experimental Biology and Medicine 90, 210–213.

Xu, W.N., Li, J.Y., Li, X.F., Huang, G.Q. and Liu, W.B. (2013). Alterations of liver histology and blood biochemistry in blunt snout bream *Megalobrama amblycephala* fed high-fat diets. Fisheries Science 79, 661-671.

Xu, X., Yang, H., Zhang, C., Bian, Y., Yao, W., Xu, Z., Wang, Y., Li, X. and Leng, X. (2022). Effects of replacing fishmeal with cottonseed protein concentrate on growth performance, flesh quality and gossypol deposition of largemouth bass (*Micropterus salmoides*). Aquaculture 548, p.737551.

Yan, Q.G., Zhu, X.M., Yang, Y.X., Han, D., Jin, J.Y., Xie, S.W. and Li, Z.J. (2014). Effect of replacement of fish meal with cottonseed meal on growth, hematological physiology, and body composition of grass carp. Acta Hydrobiologica Sinica 38, 362-369. (abstract in English).

Yang, Y., Xie, S., Lei, W., Zhu, X. and Yang, Y. (2004). Effect of replacement of fish meal by meat and bone meal and poultry by-product meal in diets on the growth and immune response of *Macrobrachium nipponense*. Fish & Shellfish Immunology 17, 105-114.

Yao, W., Zhang, C. and Li, X. (2020). The replacement of fish meal with fermented soya bean meal or soya bean meal in the diet of Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture Research, https:// doi.org/10.1111/are.14583

Yildirim, M., Lim, C., Wan, P.J. and Klesius, P.H. (2003). Growth performance and immune response of channel catfish (*Ictalurus punctatus*) fed diets containing graded levels of gossypol-acetic acid. Aquaculture 219, 751-768.

Yue, Y.R. and Zhou, Q.C. (2008). Effect of replacing soybean meal with cottonseed meal on growth, feed utilization, and hematological indexes for juvenile hybrid tilapia, *Oreochromis niloticus* × *O. aureus*. Aquaculture 284, 185–189.

Zhang, W.J., Xu, Z.R., Zhao, S.H., Sun, J.Y. and Yang, X. (2007). Development of a microbial fermentation process for detoxification of gossypol in cottonseed meal. Animal Feed Science and Technology 135, 176–186.

Zhao, W., Liu, Z.L. and Niu, J. (2021). Growth performance, intestinal histomorphology, body composition, hematological and antioxidant parameters of *Oncorhynchus mykiss* were not detrimentally affected by replacement of fish meal with concentrated dephenolization cottonseed protein. Aquaculture Reports 19, 100557.

Zheng, Q., Wen, X., Han, C., Li, H. and Xie, X. (2012). Effect of replacing soybean meal with cottonseed meal on growth, hematology, antioxidant enzymes activity and expression for juvenile grass carp, *Ctenopharyngodon idellus*. Fish physiology and biochemistry 38, 1059-1069.

LIST OF MANUSCRIPT PUBLISHED

1. A manuscript entitled "**Dietary free gossypol isolated from cottonseed meal on hematological and biochemical responses of snubnose pompano, *Trachinotus blochii* (Lacepède 1801)"** has been published in Journal of Experimental Zoology, India.

2. A manuscript entitled "**An alternate protein source for fishmeal: Cottonseed protein concentrate production optimization and its nutritive profile"** has been published in Indian Journal of Fisheries.

3. A manuscript entitled " **Enzymological, histological, and serum biomarker responses of snubnose pompano on complete replacement of fishmeal using cottonseed meal supplemented with lysine and methionine in the diet"** has been published in Fish Physiology and Biochemistry.

Abstract presented

1. Participated and presented an oral presentation on "**Fish meal replacement with cottonseed meal on growth, feed utilization and hemato-immunological response of snubnose pompano**" during the **International conference on Aquatic Resources and Blue Economy (AQUABE2019)** at Kerala University of Fisheries and Ocean Studies (KUFOS), Kochi, Kerala held during 28-30th November 2019.

2. Participated and presented an oral presentation on "**Dietary free gossypol on hematological, biochemical and survival of snubnose pompano, *Trachinotus blochii* (Lacepède 1801) challenged with *Vibrio parahaemolyticus"*** during the 3rd International Conference on Aquaculture and Marine Biology organized by Conference Mind at Goa during 24-25th March 2022.

3. Participated and presented an oral presentation on "**Cottonseed protein concentrate on growth, digestive enzymology and antioxidant defence response in snubnose pompano"** during the 3rd International Conference on Aquaculture and Marine Biology organized by Conference Mind at Goa during 24-25th March 2022.

4. Participated and presented an oral presentation on **"Fishmeal replacement using cottonseed meal on growth, digestive enzymology and muscular free gossypol in Snubnose pompano, *Trachinotus blochii*: An economic appraisal"** during the 12th Indian Fisheries and Aquaculture Forum organized by Tamil Nadu Dr. J. Jeyalalitha Fisheries University during 5-7th May 2022 at Chennai.

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DIETARY FREE GOSSYPOL ISOLATED FROM COTTONSEED MEAL ON HEMATOLOGICAL AND BIOCHEMICAL RESPONSES OF SNUBNOSE POMPANO, *TRACHINOTUS BLOCHII* (LACEPÈDE 1801)

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ABSTRACT : A 45-day feeding experiment was conducted to determine the effect of dietary free gossypol on hematological and biochemical response of snubnose pompano, *Trachinotus blochii*. Fishes with the average weight of 11.43 ±0.25 g were randomly stocked in five treatments each with triplicates. Five isonitrogenous and isolipidic diets with graded level of free gossypol (0, 0.2, 0.5, 1 and 2 g/kg) were formulated and fed to respective treatments. There was significant difference (P<0.05) in moisture, ether extract, total ash and nitrogen free extract content among the treatments. There was no significant difference (P>0.05) in the hematological response among the treatments. Higher levels of hemoglobin, RBC, HCT and MCHC values were observed in control group. Lipid biochemical profile in terms of total lipid, triglycerides and very low density lipoprotein showed significant difference (P<0.05) among the treatments. However, serum cholesterol, phospholipids, high density lipoprotein and low density lipoprotein levels did not showed significant difference among the treatments (P>0.05). As a conclusion, based on the second degree polynomial regression analysis of serum lipid biochemical parameters such as total lipids and high density lipoprotein levels indicates that snubnose pompano can tolerate upto 1.2 g of free gossypol per kg diet without any adverse effect on general health.

Key words : Free gossypol, Snubnose pompano, lipid profile, hematological response, cottonseed meal.

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INTRODUCTION

Fishmeal is one of the most indispensable feed ingredients in marine fish diet due to its short supply, high demand and elevated price. Therefore, there is a need to search for an alternate feed ingredient to replace fishmeal in marine fish feeds (Fournier *et al*, 2004). Among the several feed ingredients available, cottonseed meal (CSM) is one of the most promising alternatives to fishmeal due to its highly digestible protein content, adequate availability, high palatability and practically low price (Li and Robinson, 2006). However, CSM contains some anti-nutritional factors (ANFs) namely free gossypol, cyclopropene fatty acids, phytic acid and tannin. Among the ANFs of cottonseed products, free gossypol is a most notorious one in monogastric animals including fish species. Gossypol is a yellow pigmented phenolic compound found mainly in the seeds of cotton plant and by the way found in cottonseed products such as CSM and oil cakes (Dodou, 2005). Gossypol is found in two forms namely free and bound forms in the cottonseed products (Kenar, 2006). The bound form of gossypol is produced through the covalent bonds between free gossypol and free epsilon-amino groups of lysine and arginine that affects the bioavailability of these amino acids to the animals (Fernandez *et al*, 1995).

Some studies stated that free gossypol content have deleterious effect on feed intake, growth, feed efficiency, gut health and general physiology in fish (Anderson *et al*, 2016; Liu *et al*, 2020). Yildirim *et al* (2003) found that dietary gossypol concentrations of 0-1500 mg/kg in the diet of juvenile channel catfish for 12 weeks revealed that 300 mg/kg diet and above are toxic in terms of the reduction in body lipids, proteins and red blood cell count; however survival was not affected. Barros *et al* (2002) revealed that in juveniles of *Ictalurus puctatus* replacement of soybean meal using cottonseed meal by

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Cottonseed protein concentrate as an alternate protein source for fishmeal replacement in aquafeeds: Production optimisation and its nutritive profile

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ABSTRACT

Cottonseed protein concentrates (CPC) was produced from extruded cottonseed meal (CSM) through iso-electric pH precipitation method and the production protocol was optimised for maximum yield of CPC. The processing characteristics such as yield, protein content and key functional properties were investigated. The average yield of CPC obtained was 28.35%. Protein content of CPC was 69.47±1.28% which showed 64.97% increase in protein content of CSM used for production. Amino acid profile of CPC was on par with fishmeal for most of the essential amino acids expect for lysine and methionine. Functional properties such as water holding capacity and oil holding capacity of CPC was not statistically different from fishmeal ($p>0.05$) and other properties of CPC such as foaming capacity, foaming stability, heat coagulated protein and bulk density were significantly different ($p<0.05$) from fishmeal. The CPC production process significantly reduced the free gossypol content of CSM to the extent of 79.73% which will help to alleviate the detrimental effects of CSM. Pepsin digestibility of CPC was significantly higher than CSM and slightly lower than FM. The results of this study revealed that CPC could be a suitable alternative protein source feed ingredient for replacement of fishmeal in the diet of marine fishes due to its higher digestible protein, superior amino acid profile and lower gossypol content.

Keywords: Bulk density, Cottonseed protein concentrate, Foaming capacity, Free gossypol content, Pepsin diigestibility

Introduction

Aquafeeds contain higher amount of fishmeal (FM) than feeds of terrestrial animals. The inclusion level of fishmeal in aquafeed varies according to the feeding nature whether carnivorous or omnivorous and the pattern of utilisation of nutrients (Martin, 1999). Fishmeal is one of the most indispensable ingredients in marine fish feeds. As FM is the scarcest ingredient due to its decreased supply, high demand and price; there is a search for an alternate ingredient to replace FM in marine fish diets (Fournier *et al*., 2004). Among the several feed ingredients available in the market, agricultural byproducts or plant protein sources are widely available, renewable and reasonably priced ingredients (Chakraborty *et al*., 2019). Therefore, the use of plant protein sources in aquafeeds is considered as potential alternatives to fishmeal (Rana *et al*., 2009). Unfortunately, plant source proteins are often deficient in certain essential amino acids and boast high amounts of fibre content, complex carbohydrates, non-starch polysaccharides and presence of anti-nutritional factors (ANFs), which cause adverse effects on feed intake, digestion and absorption leading to reduced growth (Hixson, 2014). The ANFs may also have adverse effects on both their nutritional value and palatability (Kaushik *et al*., 2004). Hence, an elucidation to ameliorate the above mentioned issues to an acceptable level for inclusion of agricultural byproducts in place of fishmeal in marine fish feeds is warranted.

Among the different plant source proteins, cottonseed meal (CSM) is one of the most promising alternatives to marine source ingredients due to its high protein content (42-45%), wide availability, palatability and reasonably low price (Li and Robinson, 2006). Like other ingredients of plant origin, cottonseed meal also contains some ANFs such as gossypol, cyclopropene fatty acids, phytic acid and tannin. In aquafeeds, cottonseed meal is used to replace soybean meal and fishmeal due to its better palatability and nutritional quality (Li and Robinson, 2006). Some studies stated that cottonseed meal has deleterious effect on feed intake, growth, feed efficiency, gut health and general physiology of fish (Lee *et al.,* 2006; Anderson *et al.,* 2016; Liu *et al.,* 2020). Therefore, it is necessary to ameliorate the ill effects of cottonseed meal. Removal of anti-nutritional factors from feed ingredients is a cumbersome work and most of the methods employed for eradication of anti-nutritional factors in small scale

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Enzymological, histological, and serum biomarker responses of snubnose pompano on complete replacement of fishmeal using cottonseed meal supplemented with lysine and methionine in the diet

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Abstract In a feeding experiment, cottonseed meal (CSM) was used to replace fishmeal (FM) in the diet of snubnose pompano, *Trachinotus blochii*, supplemented with lysine and methionine to assess the growth, nutritive profile, hematological, histological, and stress biomarker response. Experimental fishes were randomly stocked in five treatments each with triplicates. Five isonitrogenous and isolipidic diets with graded level of CSM (0, 8.7, 17.4, 26.0, and 34.7%) as replacement for FM protein (0, 25, 50, 75, and 100%) were formulated and fed to respective treatments. Comparison between various parameters among the treatments was made using orthogonal polynomial contrasts to indicate the statistical significance. Higher alkaline phosphatase, acid phosphatase, lactate dehydrogenase, malate dehydrogenase, aspartate, and alanine aminotransferase activities were observed in 0CSM group and followed by 100CSM group as higher inclusion level of CSM with higher free gossypol content did not affect the metabolic enzyme activities. The maximum muscular free gossypol accretion of 1.28 mg kg^{-1} (on wet basis) was recorded in 100CSM group which was very well below the critical limit set by FDA. As a conclusion, fishmeal can be completely replaced using cottonseed meal in the diet of pompano without adverse effect on growth, metabolism, and general health.

Keywords Unconventional feed ingredient · *Trachinotus blochii* · Fishmeal replacement · Free gossypol accretion · Lysine deficiency · Sustainable resource

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Introduction

As the global fish production steadily growing, fish feed production is increasing tremendously. Even though aquafeed production goes up, cost-effective feed is not available in the market, especially for carnivorous marine fishes. The reason behind such situation is higher inclusion level of fishmeal (FM) and fish oil in the diet of carnivorous marine fishes. Thus, fishmeal became one of the most indispensable components in marine fish feeds, and its requirement is elevating exponentially. But, fishmeal production is almost static around 5 Mt (Bachis 2017; FAO 2020) in which 69% is used by aquaculture industry alone (Bachis 2017). However, unfortunately there is no scope to escalate the FM production in the future and the FM price is inflating steadily. Therefore, utilization of FM in marine carnivorous fish feeds has started declining over the years (FAO 2020; Naylor et al. 2021). The increased demand of FM coupled with rise in prices led to

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Fishmeal replacement with cottonseed meal on growth, feed utilization and hemato-immunological response of snubnose pompano

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An experiment was conducted to evaluate the effect of fishmeal replacement with cottonseed meal (CSM) on growth, feed utilization and hemato-immunological response of snubnose pompano, *Trachinotus blochii*. After acclimation, the fishes with the average weight of 12 ±1 g were stocked in five different treatments such as control diet (C), 25% FM replaced with CSM (25CSM), 50% FM replaced with CSM (50CSM), 75% FM replaced with CSM (75CSM) and 100% FM replaced with CSM (100CSM) fed groups each with triplicates. The experiment was conducted for 10 weeks. The growth, feed utilization, metabolism and haemato-immunological parameters were studied among the treatments. Better growth in terms of weight gain percentage and specific growth rate was noticed in control diet fed groups (385.68% and 2.25%) followed by 100 CSM group (368.3% and 2.20%). Better FCR was witnessed in 1:1.26 was found in 100CSM group. The histological observation of liver samples indicates that 100% CSM replacement showed some vacuoles in the hepatocytes while comparing with 0% CSM diet. Higher hemoglobin content, WBC and RBC count was witnessed in 50 CSM group. Higher MCV was observed in control group and higher MCH and MCHC values were noticed in 25 CSM group. The whole body proximate composition of experimental animals revealed that total ash content was increasing as the CSM inclusion increased and reverse trend was observed for gross energy content of the tissue samples. This study reveals that fishmeal can be replaced completely with CSM without any adverse effect on growth and general well-being of snubnose pompano.

Cottonseed protein concentrate on growth, digestive enzymoloy and antioxidant defence response in snubnose pompano

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Abstract:

A 10 week feeding trial was conducted to elucidate the effect of cottonseed protein concentrate (CPC) on growth, hematology, intestinal histology, and antioxidant defence system response of Trachinotusblochii. The fishes with the average weight of 4.05 ±0.3 g were stocked in five different treatments (0CPC, 25CPC, 50CPC, 75CPC and 100CPC) each with triplicates.Five iso-nitrogenous (40% CP) and iso-caloric (6% CL) experimental feeds with varying level of CPC incorporation by substituting fishmeal were prepared. The growth performance of fish fed with graded levels of CPC showed significant difference (P<0.05) in weight gain percentage (WG%), specific growth rate (SGR), average daily growth (ADG), thermal growth co-efficient (TGC) and daily growth co-efficient (DGC) and higher values were observed in 0CPC group followed by 25CPC group.Among the treatments, better feed conversion ratio (FCR), protein efficiency ratio (PER), feed efficiency (FE), protein productive value (PPV), energy retention efficiency (ERE) and nitrogen retention efficiency (NRE) were observed in 25CPC group followed by 0CPC group. The intestinal protease activity showed significant difference (P<0.05) among the treatments and higher protease activity was witnessed in 0CSP group and the activity was decreased as the level of inclusion of CPC increased in the diet. There was no significant difference (P>0.05) in the α-amylase and lipase activities among the treatments. The antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and Glutathione-S-transferase (GST) showed a significant difference (P<0.05) in different tissues among the treatments. As the inclusion of CPC increased in the diet, the SOD, CAT and GST levels were gradually increased, and a higher level was recorded in 100CPC group. The second degree polynomial regression analysis of growth, biochemical and enzymological parameters revealed that CPC can be included in the range of 8.29-17.58% of diet which accounts 27.63-58.60 % of fishmeal replacement in the diet of snubnose pompano.

Keywords: Cottonseed protein concentrate; hi-protein feed ingredient; antioxidant defence; marine fish nutrition

Biography:

D. Linga Prabu is an Agricultural Research Service-Scientist at ICAR-Tuticorin Regional Station of Central Marine Fisheries Research Institute, Thoothukudi and he is mainly engaged in marine fish nutrition research.

Dietary free gossypol onhematological,biochemical and survival of snubnosepompano, Trachinotusblochii(Lacepède 1801) challenged with Vibrio parahaemolyticus

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Abstract:

A 45-day feeding experiment was conducted to determine the effect of dietary free gossypol on hematological, biochemical response and survival of challenged with V. parahaemolyticus. Fishes with the average weight of 11.43 ±0.25 g were randomly stocked in five treatments each with triplicates. Five isonitrogenous and isolipidic diets with graded level of free gossypol (0, 0.2, 0.5, 1 and 2 g/kg) were formulated and fed to respective treatments (0G, 0.2G, 0.5G,1G and 2G). After completion of feeding trial and sampling, 8fishes from each replicate of the same treatments were experimentally infected intra-peritoneallyusing 0.1 mL of pathogenic Vibrioparahaemolyticusat a dosage of 1.0×107 CFU mL-1. The fishes of each treatment group were injected. There was no significant difference (P>0.05) in the hematological response among the treatments. Higher levels of hemoglobin, RBC, HCT and MCHC values were observed in control group. Lipid biochemical profile in terms of total lipid, triglycerides and very low density lipoprotein showed significant difference (P<0.05) among the treatments. However, serum cholesterol, phospholipids, high density lipoprotein and low density lipoprotein levels did not showed significant difference among the treatments (P>0.05). Among the different treatments better relative percentage of survival was observed in 0.2G groupand followed by 0G group. No significant difference (P>0.05) in relative percentage of survival and mortality rate of snubnose pompano among the treatments. As a conclusion, based on the second degree polynomial regression analysis snubnose pompano can tolerate up to 1.2 g of free gossypol per kg diet without any adverse effect on general health.

Biography

D. Linga Prabu is an Agricultural Research Service-Scientist at ICAR-Tuticorin Regional Station of Central Marine Fisheries Research Institute, Thoothukudi and he is mainly engaged in marine fish nutrition research.

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Fishmeal Replacement Using Cottonseed Meal on Growth, Digestive Enzymology and Muscular Free Gossypol in Snubnose Pompano, *Trachinotus blochii*: An Economic Appraisal

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A 10 week study was conducted in indigenous re-circulatory aquaculture systems to evaluate the effect of fishmeal replacement using cottonseed meal (CSM) on growth, digestive enzymology and feed economics in snubnose pompano, *Trachinotus blochii*. Five isonitrogenous and isolipidic diets with graded level of CSM (0, 8.7, 17.4, 26.0 and 34.7%) as replacement for FM protein (0, 25, 50, 75, and 100%) were formulated and fed to respective treatments (0CSM, 25CSM, 50CSM, 75CSM and 100CSM). The growth response in terms of weight gain percentage (WG%), specific growth rate (SGR), average daily growth (ADG), thermal growth co-efficient (TGC), geometric mean body weight (W_G) and growth rate on metabolic body weight (GR_{MBW}) were assessed and found higher in 0CSM group followed by 100CSM group. Feed intake on % of body weight (FI_{PCT}), feed intake per metabolic body weight (FI_{MBW}) and protein productive value (PPV) showed significant difference ($P<0.05$). Lower FI_{PCT} and FI_{MBW} were observed in 25CSM group, followed by 50CSM group. Among the treatments, better FCR was observed in 100CSM group and better PER and PPV were witnessed in 0CSM group. The intestinal α-amylase, protease and lipase activities showed significant difference ($P<0.05$) among the treatments. The maximum protease and lipase activity were noticed in 0CSM treatment and α-amylase activity was higher in 50CSM treatment. The maximum muscular free gossypol accretion of 1.28 mg kg^{-1} (on wet basis) was recorded in 100CSM group which was very well below the critical limit set by FDA. The relative feed cost of 100CSM diet was 10.08% lower than 0CSM diet. Higher relative feed cost savings of 10.79% was witnessed in 100CSM group. As a conclusion, fishmeal can be completely replaced using cottonseed meal in the diet of pompano without adverse effect on growth, metabolism, meat quality and general health with adequate savings of production cost

Keywords : *Trachinotus blochii*, Sustainable Resource, Palatable Feed Ingredient, Cheaper Protein, Economical

CERTIFICATES

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IN ASSOCIATION WITH LEADING NATIONAL ORGANISATIONS

CERTIFICATE

This is to certify that D. Linga Prabhu

participated in the International Conference on Aquatic Resources and Blue Economy 2019 organized by Kerala University of Fisheries and Ocean Studies held from November 28th to 30th 2019 and presented a paper (Oral) entitled Fish meal replacement with cotton seed meal ..... Amblypes pompano authored by D. Linga Prabhu, P. Vijaya Gopal, C. Kalidas, M. Kavitha, P.P. Manoj Kumar

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CERTIFICATE OF RECOGNITION

AQUACULTURE 2022

Is hereby presented to

D. Linga Prabu

(Agricultural Research Service-Scientist, ICAR-Tuticorin Regional Station of Central Marine Fisheries Research Institute, India)

For sharing his valuable knowledge as a Speaker with the oral presentation entitled "Cotton seed protein concentrate on growth, digestive enzymoloy and antioxidant defence response in snubnose pompano" at the occasion of the 3rd International Conference on Aquaculture & Marine Biology, during March 24-25, 2022

Sanjay Kumar Gupta
Senior Scientist, ICAR-IIAB, Ranchi, India
(SESSION CHAIR)

Dr. Emmanuel Parlier
Founder and Owner of "Parlier Environment SAS" Legal expert and Aquaculture Consultant, France

Binay Kumar Chakraborty
Project Director at Department of Fisheries, Bangladesh Agricultural University, Bangladesh
(Organizing Committee Member)

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12th INDIAN FISHERIES & AQUACULTURE FORUM

5-7 MAY 2022, CHENNAI

Certificate

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This is to certify that **D LINGA PRABU** participated as a registered delegate in the **12th Indian Fisheries & Aquaculture Forum** organized by **Tamil Nadu Dr. J Jayalalithaa Fisheries University (TNJFU) & Asian Fisheries Society Indian Branch (AFSIB)** during **5-7 May 2022** and presented a paper entitled **FISHMEAL REPLACEMENT USING COTTONSEED MEAL ON GROWTH, DIGESTIVE ENZYMOLOGY AND MUSCULAR FREE GOSSYPOL IN SNUBNOSE POMPANO, TRACHINOTUS BLOCHII: AN ECONOMIC APPRAISAL.**

Dr J STEPHEN SAMPATH KUMAR
ORGANIZING SECRETARY

Dr. G SUGUMAR
CONVENER

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